UNITED STATES ENVIRONMENTAL PROTECTION AGENCY

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OFFICE OF
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AND TOXIC SUBSTANCES

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HEALTH EFFECTS DIVISION
SCIENTIFIC DATA REVIEWS
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MEMORANDUM

Date: 6-5-02

Subject: **Cyhalofop-butyl:** Review of Toxicity Studies.
DP Barcode: D268553, D267558
PC Code: 082583

To: Joanne Miller, PM Team 23
Registration Division (7505C)

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Dow AgriSciences LLC submitted an extensive toxicity data base for cyhalofop-butyl. Data Evaluation Records (DERs) for each toxicity study are attached. All studies are acceptable except for the carcinogenicity study in mice (MRID No. 45000418) and the carcinogenicity portion of the chronic feeding and carcinogenicity study in rats (MRID No. 45000417). The doses in these two studies were inadequate to assess the carcinogenic potential of cyhalofop-butyl. These carcinogenicity studies, along with a 28-day inhalation toxicity study, have been identified as data gaps, and new studies have been requested.

The following is a hazard profile for cyhalofop-butyl, followed by a toxicity profile which describes the status of the cyhalofop-butyl data base and the findings for each study.

Toxicity Data Base Overview: An extensive toxicity data base has been provided for cyhalofop-butyl. Oral and dermal toxicity have been well characterized, but inhalation toxicity has not. The low toxicity of this herbicide is evident from the fact that many studies were performed at limit doses, usually with no toxicity. The carcinogenic potential of cyhalofop-butyl has not been characterized because the doses tested in the rat and mouse carcinogenicity studies were too low.

Acute Toxicity: The Toxicity Category for the technical is **IV** for oral, dermal, and inhalation toxicity, and for eye and skin irritation. Cyhalofop-butyl is not a dermal sensitizer.

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Chemical:	**Propanoic acid, 2-?4-(4-cyano-2-fluoroph**
PC Code:	**082583**
HED File Code	**13000 Tox Reviews**
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HED Records Reference Center
11/04/2002

Route-Specific Toxicity: Toxicity by the oral route has been well characterized in the rat, mouse, rabbit, and dog. A 21-day dermal toxicity study in rats revealed no evidence of toxicity at the limit dose of 1000 mg/kg/day. Aside from an acute toxicity study, there are no inhalation toxicity data with which to perform a route-specific risk assessment. Extrapolating from the oral route will likely underestimate the inhalation risk.

Pharmacokinetics and Cumulative Toxicity: The pharmacokinetics of cyhalofop-butyl has been well explored in rats, mice, rabbits, and dogs. A dermal penetration study in rats demonstrated absorption of various cyhalofop-butyl dilutions to be 25-34% following a 24-hour dermal dosing, with >85% of the dose being excreted in the urine and <1% being excreted in the feces within 48 hours.

A metabolism and pharmacokinetic study of labeled and nonlabeled cyhalofop-butyl in rats demonstrated that absorption of gavaged test article was 93-100% with urinary excretion being the major route of elimination regardless of dose, label position, or gender. Over 168-hours, 84-100% of the radioactivity was eliminated via the urine, with 86-90% eliminated within 24 hours. Fecal excretion was <5%. There was no elimination via expired air. Over a 24-hour period, biliary elimination accounted for 1.7 % and 20.1% of the administered dose in males and females, respectively, in the low-dose [α-^{14}C]XRD-537 BE group, and 17.0% (males) and 11.6% (females) of the administered dose in the [β-^{14}C]XRD-537 BE low-dose group. The greatest radioactivity levels were found in the liver, kidneys, plasma, whole blood, heart, lung, and stomach, with the highest tissue levels being found in the liver and kidney at 2 hours. Most tissue levels accounted for <1% of the administered dose. Due to rapid excretion, tissue/organ levels declined to near detection limits by 24 hours in all dose groups. There was a biphasic pattern for both labels with no substantial differences in pharmacokinetic indices (C_{max}, t_{cmax}, $t_{1/2}$, AUC). Time-to-maximum plasma concentration (t_{cmax} of 0.5 to 4 hrs) and elimination half-times ($t_{1/2}$ of 1.4-7.9 hrs) reflected the relatively rapid absorption. Females had somewhat shorter t_{cmax} and lower C_{max} values suggestive of saturated absorption processes. The acid metabolite (R-(+)-2-[4-(4-cyano-2-fluorophenoxy)phenoxy]propanoic acid) was the most prominent plasma fraction (~90-94% of the plasma activity for males and ~75-81% for females regardless of dose). No parent compound or other metabolites were detected. The acid metabolite was the most common product in urine and feces–71-87% (urine) and 46-75% (feces) of the activity in those matrices.

Although many acute and subacute studies were performed at limit doses, often with no toxicity, the relatively low subchronic and chronic NOAELs show that moderate systemic cumulation occurs.

Subacute and Subchronic Toxicity: No adverse toxicity was observed in the 21-day dermal toxicity study. Liver weights were elevated, but returned to normal in a recovery group, as might be expected for an adaptive response.

Subchronic feeding NOAELs in Sprague-Dawley rats were considerably higher than in Fischer rats. The LOAELs were based on perineal soiling, and reduced body weights and body weight gain in Sprague-Dawley females, and on lipofuscin pigment deposition in proximal tubule cells in Fischer rats of both sexes.

Two subchronic feeding studies were performed in CD-1 mice with different results. No toxicity was observed in males or females in one study. In the other study, enlarged kidneys (20% absolute and relative) and swelling of the proximal tubule cells were observed in 4 of 12 females at a relative low dose.

In a subchronic feeding study in dogs, brown and/or atrophied thymuses, and decreased thymus weights were observed in males and females. This is the only study in which thymus effects were observed.

Developmental and Reproductive Toxicity: No maternal or developmental toxicity was seen in rats when the dams were dosed by gavage up to the limit dose. No developmental effects were seen in rabbit pups when the does were dosed up to the limit dose, but maternal deaths and "cloudy" or dark colored kidneys were observed at lower doses.

In a two generation reproductive toxicity study in Crj:CD (SD) rats, slight renal tubular cell swelling was observed in F_0 and F_1 male rats, but not in females. There was no effect on reproduction, and there were no developmental effects observed in the pups.

Chronic Toxicity/Carcinogenicity and Mutagenicity:

Chronic effects observed in Fischer rats included early and increased deposition of the pigments lipofuscin and hemosiderin in the renal proximal tubular cells of both sexes and renal mineralization in females at the highest dose tested. Chronic effects in CD-1 mice included tubular dilatation, chronic glomerulonephritis, and hyaline casts in females, and hyperplasia of the stomach mucosal epithelium in males at the highest dose tested. No chronic toxicity was observed in a dog study at the doses tested.

The HIARC determined that the doses used in the rat and mouse carcinogenicity studies were not adequate to assess the carcinogenic potential of cyhalofop-butyl. There was no evidence of carcinogenicity in either species. Five mutagenicity studies had negative results including an Ames assay, a gene mutation assay in mouse lymphoma L5178Y TK cells, an *in vivo* micronucleus assay in mouse bone marrow cells, an unscheduled DNA synthesis assay in rat hepatocytes, and an *in vitro* chromosomal aberration assay in Chinese hamster lung.

Neurotoxicity: There is no evidence of neurotoxicity in any studies, including an acute gavage neurotoxicity study in rats (performed at the limit dose) and a subchronic feeding neurotoxicity study in rats. The doses used in the subchronic study were too low to elicit any toxicity, however.

Endocrinopathy: None of the animal studies provide any evidence that cyhalofop-butyl disrupts endocrine receptors.

Table 1 presents the acute toxicity of cyhalofop-butyl. **Table 2** is a toxicity profile which describes the status of the cyhalofop-butyl data base and the findings for each study.

Table 1. Acute Toxicity of Cyhalofop-Butyl Technical

OPPTS No./Study Type	MRID	Results
870.1100 Acute Oral - Rat Kodaira Laboratories Study No. GHF-R 260 January 13, 1992	45000237	**LD_{50} >5000 mg/kg (limit test)** There was no evidence of toxicity. **Toxicity Category IV** Acceptable/Guideline
870.1100 Acute Oral - Mice Kodaira Laboratories Study No. GHF-R 258 January 13, 1992	45000238	**LD_{50} >5000 mg/kg (limit test)** There was no evidence of toxicity. **Toxicity Category IV** Acceptable/Guideline
870.1200 Acute Dermal - Rat Kodaira Laboratories Study No. GHF-R 259 January 13, 1992	45000240	**LD_{50} >2000 mg/kg (limit test)** There was no evidence of toxicity. **Toxicity Category III** Acceptable/Guideline
870.1200 Acute Dermal - Rat Health & Environmental Research Laboratories, Dow Chemical Co. Study No. 981083 July 6, 1998	45381901 45000241	**LD_{50} >5000 mg/kg (2.5 x the limit dose)** Chromodacryorrhea was observed in 2/5 males on day 2 only. Delayed weight gain was observed in all rats, with the females being most affected. There was no dermal irritation. **Toxicity Category IV** Acceptable/Guideline
870.1300 Acute Inhalation - Rat Mitsukaido Laboratories Study No. IET 90-0179 January 17, 1994	45000401	**LC_{50} >5.63 mg/L (2.8 x the limit concentration)** Bradypnea was noted in all rats with recovery within two hours following exposure. Abnormal respiratory sounds were noted in all rats after exposure with recovery by day 1. Reddish adhesive materials in the nasorostral and periocular regions were noted from all test rats after exposure with recovery by day 2. No gross abnormalities. Two control rats had reddish adhesive materials in the nasorostral region after exposure with recovery within two hours. **Toxicity Category IV** Acceptable/Guideline
870.2400 Primary Eye Irritation - Rabbit Safepharm Laboratories Ltd.; Study No. 413/9 October 20, 1993	45000403	Minimally irritating **Toxicity Category IV** Acceptable/Guideline

OPPTS No./Study Type	MRID	Results
870.2500 Primary Skin Irritation - Rabbit Safepharm Laboratories Ltd.; Study No. 413/8 October 20, 1993	45000405	Essentially nonirritating **Toxicity Category IV** Acceptable/Guideline
870.2600 Dermal Sensitization - Guinea Pig Huntingdon Life Sciences Ltd. Study No. GHE-T-591 January 11, 1996	45000407	Not a dermal sensitizer Acceptable/Guideline

Table 2. Toxicity Profile of Cyhalofop-Butyl Technical

OPPTS No./Study Type	MRID	Results
870.3100 Subchronic (4 and 13 Week) Feeding - Rat Toxicology Research Laboratory, Dow Chemical Co. Study Nos. DR-0298-8876-003 and DR-0298-8876-003A March 7, 1991	45000413	**NOAEL (male) ≥400 mg/kg/day (HDT in ♂)** **NOAEL (female) 400 mg/kg/day** **LOAEL (female) = 800 mg/kg/day (HDT in ♀) based on perineal soiling and reduced body weights and body weight gain.** The only short-term effect was perineal staining. Onset was late except in 60% of the 800 mg/kg/day females which had involvement as early as day 16. The only functional observation battery (FOB) finding was perineal soiling at the high-dose in 1/10 males and 6/10 females. In addition to the 13 week study, which included a FOB, a 4 week satellite was used to determine organ weight and microscopic changes in potential target tissues. *Levels tested:* Main - 0, 3 (males), 10 (females), 25 (males), 100, 400, or 800 (females) mg/kg/day in the feed for 13 weeks in Sprague-Dawley strain. Satellite - 0, 25, 400 (males), 800 (females), or 1600 mg/kg/day in the feed for 4 weeks. Acceptable/Guideline
870.3100 Subchronic Feeding - Rat Mitsukaido Laboratories Study No. GHF-P-1385 March 3, 1993	45014705	**NOAEL = 60.5 / 65.3 mg/kg/day, M/F** **LOAEL = 189.5 / 199.6 mg/kg/day, M/F (HDT) based *on kidney toxicity (lipofuscin pigment* deposition in proximal tubule cells) in both sexes, and possible liver toxicity (hepatocyte eosinophilic granules) in males.** No short-term effects were observed which could be used for a short-term endpoint. Levels tested: 0, 30, 300, 1000, or 3000 ppm in the feed (0, 1.719, 17.43, 60.5, or 189.5 mg/ kg/day in males; 0, 1.958, 19.64, 65.3, or 199.6 mg/kg/day in females) in Fischer strain. Acceptable/Guideline

OPPTS No./Study Type	MRID	Results
870.3100 Subchronic Feeding - Mice Toxicology Research Laboratory, Dow Chemical Co. Study Nos. DR-0298-8876-002 and DR-0298-8876-002A March 7, 1991	45000412	**NOAEL (male) ≥30 mg/kg/day (HDT in ♂)** **NOAEL (female) ≥100 mg/kg/day (HDT in ♀)** Levels tested (main): 0, 1 (males), 3, 10, 30, or 100 (females) mg/kg/day in the feed. Levels tested (pilot): 0, 10 (males), 30, 100, or 350 (females) mg/kg/day in the feed. Acceptable/Guideline
870.3100 Subchronic Feeding - Mice Institute of Environmental Toxicology Study No. GHF-P-1390 March 3, 1993	45014706	**NOAEL (male) ≥37.5 mg/kg/day (HDT)** **NOAEL (female) = 4.3 mg/kg/day** **LOAEL (female) = 14.1 mg/kg/day based on enlarged kidneys (20% absolute and relative) accompanied by swelling of the proximal tubule cells (4/12 mice).** Kidney weights and pathology were normal in males. Compared to controls, absolute and (relative) kidney weight increases in females were 1, 14, 20, and 23% (-1, 12, 20, and 18%); and proximal tubular cell swelling incidences were 0/12, 0/12, 0/12, 4/12, and 6/12 at doses of 0, 3, 30, 100, and 300 ppm, respectively. No short-term effects were observed which could be used for a short-term endpoint. Levels tested: 0, 3, 30, 100, or 300 ppm in the diet (0, 0.4, 3.6, 12.4, or 37.5 mg/kg/day in males; 0, 0.4, 4.3, 14.1, or 41.4 mg/kg/day in females) Acceptable/Guideline
870.3150 Subchronic Feeding - Dog Institute of Environmental Toxicology Study No. GHF-P-1389 February 1, 1994	45014707 (main) 45000410 (pilot, palatab.)	**NOAEL = 14.7 / 15.6 mg/kg/day, M/F** **LOAEL = 75.2 / 79.4 mg/kg/day, M/F (HDT) based on brown and/or atrophied thymuses, and decreased thymus weight.** Levels tested: 0, 100, 500, or 2500 ppm (0, 2.91, 14.7, or 75.2 mg/kg/day in males; 0, 3.17, 15.6, or 79.4 mg/kg/day in females) Acceptable/Guideline MRID 45000410 was a combination 4-week pilot toxicity and a 2 week palatability study. Doses in the palatability study were 250, 500, or 1000 mg/kg/day. At 1000 mg/kg/day, food consumption was dramatically reduced, suggesting decreased palatability of the treated diet.

OPPTS No./Study Type	MRID	Results
870.3200 21-Day Dermal - Rat Health & Environmental Research Laboratories, Dow Chemical Co. Study No. 981127 January 19, 1999	45000415	**Systemic NOAEL ≥1000 mg/kg/day (limit dose)** **Dermal NOAEL ≥1000 mg/kg/day (limit dose)** Increased liver weights and clinical chemistry changes suggestive of liver adaptation were observed during the dosing interval. The reversibility of the clinical chemistry and liver weight effects in the recovery group demonstrates these changes are biological markers of exposure, not toxicity. Levels tested: 0, 10, 100, 1000 mg/kg/day in aqueous 0.5% methylcellulose, 6 hours/day, 5 days/week for 4 weeks. A recovery group was held for a 2 week period. Acceptable/Guideline
870.3700 Gavage Developmental Toxicity - Rat Kodaira Laboratories Study No. IET 90-0173 December 21, 1992	45014709	**Maternal NOAEL =1000 mg/kg/day (limit dose)** **Developmental NOAEL ≥1000 mg/kg/day (limit dose)** Levels tested: 0, 25, 250, or 1000 mg/kg/day by gavage on gestation days 6-15. Acceptable/Guideline
870.3700 Gavage Developmental Toxicity - Rabbit The Institute of Environmental Toxicology Study No. GHF-P-1391 March 8, 1994	45014710	**Maternal NOAEL = 40 mg/kg/day** **Maternal LOAEL = 200 mg/kg/day based on maternal death** **Developmental NOAEL ≥1000 mg/kg/day (limit dose)** Levels tested: 0, 40, 200, or 1000 mg/kg/day by gavage on gestation days 6-18. Acceptable/Guideline

OPPTS No./Study Type	MRID	Results
870.3800 Feeding Reproductive Toxicity - Rat Mitsukaido Laboratories Study No. GHF-P-1388 May 23, 1994	45000419	**Systemic NOAEL (males) = 100 ppm (4.85-13.75 mg/kg/day)** **Systemic LOAEL (males) = 1000 ppm (50.0-138.7 mg/kg/day) based on kidney lesions (slight tubular cell swelling) in F_0 and F_1 male rats.** **Systemic NOAEL (females) ≥1000 ppm (69.2-147.7 mg/kg/day, HDT)** **Reproductive NOAEL ≥1000 ppm (50.1-138.7 mg/kg/day for males; 69.2-147.7 mg/kg/day for females)** **Offspring NOAEL ≥1000 ppm (50-147.7 mg/kg/day)** No short-term effects were observed which could be used for a short-term endpoint. Levels tested: Dietary levels of 0, 10, 100, or 1000 ppm (F_0 males - 0, 0.495-1.049, 4.88-10.68, or 50.0-102.9 mg/kg/day; F_1 males - 0, 0.499-1.361, 4.85-13.75, or 51.1-138.7 mg/kg/day; F_0 females - 0, 0.695-1.113, 6.75-11.13, or 69.2-113.1 mg/kg/day; F_1 females - 0, 0.750-1.430, 7.42-13.96, or 74.8-147.7 mg/kg/day) in Crj:CD (SD) strain. Acceptable/Guideline
870.4100 Chronic Feeding Toxicity - Dog Institute of Environmental Toxicology Study No. GHF-P-1386 June 20, 1994	45014708	**NOAEL ≥46.7 / 45.9 mg/kg/day; M/F (HDT)** No short-term effects were observed which could be used for a short-term endpoint. Levels tested: 0, 50, 300, or 1800 ppm in the feed (males- 0, 1.22, 7.59, and 46.7 mg/kg/day; females - 0, 1.29, 7.63, and 45.9 mg/kg/day. Acceptable/Guideline

OPPTS No./Study Type	MRID	Results
870.4200 Carcinogenicity Feeding - Mouse (18 months) Mitsukaido Laboratories Study No. GHF-P-1384 June 2, 1994	45000418	**NOAEL = 0.99 mg/kg/day** **LOAEL = 10.06 / 10.28 mg/kg/day, M/F (HDT) based on effects on the kidney including tubular dilatation, chronic glomerulonephritis, and hyaline casts in females, and hyperplasia of the stomach mucosal epithelium in males.** There was no evidence of carcinogenic potential under the conditions of this study. **Dosing was too low to elicit frank toxicity and inadequate to assess carcinogenic potential.** The high dose of approximately 10 mg/kg/day was based on the endpoint of liver hypertrophy which is an adaptive response. No short-term effects were observed which could be used for a short-term endpoint. Levels tested: 0, 3, 10, or 100 ppm (0, 0.31, 0.99, and 10.06 mg/kg/day in males; 0, 0.29, 0.99, or 10.28 mg/kg/day in females) in CD-1 strain. Satellite groups were sacrificed at 26 and 52 weeks. Unacceptable/Guideline
870.4300 Chronic Feeding Toxicity/Carcinogenicity-Rat Mitsukaido Laboratories Study No. GHF-P-1387 June 2, 1994	45000417	**NOAEL = 0.823 mg/kg/day in males and 2.475 mg/kg/day in females** **LOAEL = 3.44 mg/kg/day (HDT in males), 24.97 mg/kg/day (HDT in females) based on the early and increased deposition of the pigments *lipofuscin and hemosiderin* in the renal proximal tubular cells of both sexes, and renal mineralization in female rats.** There were no treatment-related increases in tumor incidence, compared to controls. ***Dosing was too low to elicit frank toxicity and* inadequate to assess carcinogenic potential.** No short-term effects were observed which could be used for a short-term endpoint. Levels tested: 0, 3, 6, 24, or 100 ppm (0, 0.1020, 0.2047, 0.823, or 3.44 mg/kg/day) in males; 0, 6, 60, or 600 ppm (0, 0.2451, 2.475, or 24.97 mg/kg/day) in females for 104 weeks in Fischer strain. Satellite groups were sacrificed at 13, 26, 52, and 78 weeks. Acceptable/Guideline (chronic toxicity) Unacceptable/Guideline (carcinogenicity)

OPPTS No./Study Type	MRID	Results
870.5100 Bacterial Reverse Gene Mutation Test (Ames Assay) Kodaira Laboratories Study No. GHF-R-257 July 15, 1991	45000421	Negative in *Salmonella* TA strains and *E. coli* WP2 uvrA. Acceptable/Guideline
870.5300 Gene Mutation in Mouse Lymphoma L5178Y TK Cells Huntingdon Life Sciences, Ltd. Study No. DWC 740/962438 October 3, 1996	45014711	Negative Acceptable/Guideline
870.5375 *In Vitro* Chromosomal Aberration in Chinese Hamster Lung Kodaira Laboratories Study No. GHF-R 273 May 9, 1991	45000423	Polyploidy was induced when CHL (V79) cells were treated for 48 hours in the absence of S9, but there was no clastogenic effect on DNA. Acceptable/Guideline
870.5395 *In Vivo* Mammalian Cytogenetics - Micronucleus Assay in Mouse Bone Marrow Cells Kodaira Laboratories Study No. GHF-R 271 August 26, 1991	45000422	Negative Acceptable/Guideline
870.5550 Unscheduled DNA Synthesis in Rat Hepatocytes Kodaira Laboratories Study No. GHF-R 272 March 15, 1991	45000420	Negative Acceptable/Guideline

OPPTS No./Study Type	MRID	Results
870.6200 Gavage Acute Neurotoxicity - Rats Dow Chemical Company, Health & Environmental Research Laboratories Study No. 980022 June 23, 1998	45000409	**NOAEL ≥2000 mg/kg (limit dose) based on the absence of clinical signs, a lack of effects on FOB parameters and motor activity, and the absence of neuropathologic lesions.** Levels tested: 0, 200, 600, or 2000 mg/kg by gavage Acceptable/Guideline
870.6200 Feeding Subchronic Neurotoxicity - Rats Dow Chemical Company, Health & Environmental Research Laboratories Study No. 981113 February 19, 1999	45000509	**NOAEL ≥75 ♂/ ≥250 ♀ mg/kg/day (HDT) based on the absence of clinical signs, lack of effects on FOB parameters and motor activity, and absence of neuropathologic lesions.** Levels tested: 0, 2, 20, or 75 (males) / 250 (females) mg/kg/day for 13 weeks. NOTE: The doses tested were based on mild systemic effects (perineal soiling, reduced body weights, and lipofuscin pigment deposition in proximal tubule cells) at similar doses in two subchronic feeding studies in rats, and were too low to elicit toxicity in this study. Acceptable/Guideline

OPPTS No./Study Type	MRID	Results
870.xxxx [SPECIAL STUDY] Pharmacology - Mice and Rabbits - Special Study Mitsukaido Laboratories Study No. IET-91-00118 August 25, 1992	45000424	**Mice:** A single I.P. dose of 1250 or 5000 mg/kg was lethal to all male and female mice within 24 hours. Death occurred as early as three hours at 5000 mg/kg and was preceded by behavioral and motor function abnormalities (e.g., alterations in alertness, visual placing, spontaneous activity, motor incoordination, decreased muscle tone, and compromised autonomic reflexes), some of which appeared as early as 30 minutes postdosing. Male and female mice responded similarly. **NOAEL = 78.1 mg/kg** **LOAEL = 313 mg/kg (based on minimal effects including decreased spontaneous activity, minor alterations in muscle tone, and minor changes in autonomic functions such as slight hyperthermia, and slightly decreased *respiratory rate*).** **LD ≥1250 mg/kg** Levels tested in mice: 0, 4.88, 19.5, 78.1, 313, 1250, or 5000 mg/kg as a single I.P. dose. **Rabbits:** One of three rabbits gavaged at 5000 mg/kg showed decreased spontaneous activity, prostration, decreased muscle tone, compromised autonomic reflexes, and decreased respiratory and heart rate at one day after dosing, and died on Day 4. There were no clinically significant findings in the remaining rabbits of the 5000 mg/kg dose group or any lower dose groups, and no significant effects on EKGs or blood pressure in any dosed rabbits. **NOAEL = 2500 mg/kg** **LOAEL = 5000 mg/kg (based on the response of one of three test subjects including decreased spontaneous activity, prostration, decreased muscle tone, compromised autonomic reflexes, decreased respiratory and heart rate at one day after dosing, and death on day 4).** Levels tested in rabbits: 0, 313, 1250, 2500, or 5000 mg/kg as a single gavage dose. Acceptable/Non-Guideline

OPPTS No./Study Type	MRID	Results
870.7485 Absorption, Metabolism, and Excretion - Dog Tokai Research Laboratories Study No. GHF-R-295 March 3, 1995	45000425	No treatment-related adverse effects were reported. Approximately 50% of a single gavage dose was absorbed over several hours. Blood and plasma radioactivity peaked after 1-2 hours. Clearance from plasma and blood was not especially rapid but nearly complete at 48 hours. Over 168 hours, excretion was 42.5-43.9% in the urine, and 48.6-50.6% in the feces. Tissue distribution was not measured. The test article appears to be metabolized primarily by hydrolysis to R-(+)-2-[4-cyano-2-fluorophenoxy)phenoxy]propanoic acid which was found in both the urine and feces. Several other metabolites were also formed, each representing <5% of the administered dose. No parent compound was found in the urine, and only minimal amounts were detected in the feces. Level tested: Two male beagles were gavaged with [$\propto$-^{14}C]XRD-537 BE and nonlabeled XRD-537 at a dose of 1 mg/kg. Acceptable/Non-Guideline (Two dogs were used instead of four; and tissue distribution was not measured.)

OPPTS No./Study Type	MRID	Results
870.7485 Metabolism and Pharmacokinetics - Rat Tokai Research Laboratories Study GHF-R-297 (main) March 3, 1995	45000427 (main) 45000426 (prelim.) 45000528 (stability, homog.)	Absorption of gavaged test article was 93-100%, and urinary excretion was the major route of elimination regardless of dose, label position, or gender. Over 168-hours, 84-100% of the radioactivity was eliminated in urine, with 86-90% eliminated within 24 hours. Fecal excretion was <5%. There was no elimination via expired air. Over a 24-hour period, biliary elimination accounted for 1.7 % and 20.1% of the administered dose in males and females, respectively, in the low-dose [α-^{14}C]XRD-537 BE group, and 17.0% (males) and 11.6% (females) of the administered dose in the [β-^{14}C]XRD-537 BE low-dose group. The greatest radioactivity levels were found in liver, kidneys, plasma, whole blood, heart, lung, and stomach, with the highest tissue levels being found in the liver and kidney at 2 hours. Most tissue levels accounted for <1% of the administered dose. Due to rapid excretion, tissue/organ levels declined to near detection limits by 24 hours in all dose groups. There was a biphasic pattern for both labels with no substantial differences in pharmacokinetic indices (C_{max}, t_{cmax}, $t_{1/2}$, AUC). Time-to-maximum plasma concentration (t_{cmax} of 0.5 to 4 hrs) and elimination half-times ($t_{1/2}$ of 1.4-7.9 hrs) reflected the relatively rapid absorption. Females had somewhat shorter t_{cmax} and lower C_{max} values suggestive of saturated absorption processes. The acid metabolite (R-(+)-2-[4-(4-cyano-2-fluoro-phenoxy)phenoxy]propanoic acid) was the most prominent plasma fraction (~90-94% of the plasma activity for males and ~75-81% for females regardless of dose). No parent compound or other metabolites were detected. The acid metabolite was the most common product in urine and feces–71-87% (urine) and 46-75% (feces) of the activity in those matrices. Levels tested (main study): single low dose (1 mg/kg), single high dose (50 mg/kg), and a 14-day repeated low dose (1 mg/kg/day) using non-labeled XRD-537 BE, and [α-^{14}C] XRD-537 BE or [β-^{14}C] XRD-537 BE by gavage. Acceptable/Guideline

OPPTS No./Study Type	MRID	Results
870.7600 Dermal Penetration - Rat Health & Environmental Research Laboratories, Dow Chemical Company Study No. 981090 November, 1998	45000505	Dermal absorption was ~25-34% for the spray formulation and ~11-16% for the EF-1218 formulation following a 24 hour dermal dosing. Within 48 hours, excretion was >85% in the urine and <1% in the feces, which is consistent with metabolism to water soluble metabolites and subsequent urinary excretion. Levels tested: Four Fischer 344 rats were dermally dosed for 24 hours with ^{14}C-labeled DE-537 n-butyl ester and nonlabeled DE-537 n-butyl ester in two formulations–200 mg/mL test article in EF1218 (Clincher EDC with which DE-537 n-butyl ester is normally formulated) and a spray solution–at 0.005, 1.0, or 1.8 mg/cm^2. Acceptable/Non-Guideline (Only one exposure duration (24 hours) was used instead of six.)
870.xxxx [SPECIAL STUDY] Hepatocellular Proliferation in Rats Toxicology Research Laboratory, Health and Environmental Sciences, Dow Chemical Company Study No. DR-0298-8876-008 March 4, 1991	45000414	In a subchronic oral toxicity study in rats (MRID 45000413), satellite rats dosed for 4 weeks had hepatocellular hypertrophy and focal necrosis at all dose levels. Although multiple necrotic foci accompanied by inflammatory cells were graded very slight, and were not considered dose-related, this study was performed to explore these findings. An initial dramatic increase in DNA synthesis during the first week of treatment was followed by hepatocellular hypertrophy at subsequent observations. This was the reason for enlarged livers observed in XRD-537nBu-treated rats. Levels tested: 0, 3.0, 25, 100, or 400 mg/kg/day in the diet with sacrifices at 1, 2, 4, and 13 weeks. One week prior to sacrifice, 10 µL BrdU/hour was administered via an ALZET osmotic pump implanted subcutaneously. BrdU is a DNA stain used to quantify hepatocellular proliferation. Acceptable/Non-Guideline

DATA EVALUATION RECORD

CYHALOFOP BUTYL
(XRD-537)

STUDY TYPE: ACUTE ORAL TOXICITY - RAT [870.1100 (§81-1)]
MRID 45000237

Prepared for
Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
1921 Jefferson Davis Highway
Arlington, VA 22202

Prepared by
Chemical Hazard Evaluation Group
Toxicology and Risk Analysis Section
Life Sciences Division
Oak Ridge National Laboratory
Oak Ridge, TN 37831
Task Order No. 01-81B

Primary Reviewer:
Susan Chang, M.S.

Signature: Susan Chang
Date: FEB 14 2001

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Date: FEB 14 2001

Robert H. Ross, M.S., Group Leader

Signature: Robert H. Ross
Date: FEB 14 2001

Quality Assurance:
Lee Ann Wilson, M.A.

Signature: L. A. Wilson
Date: FEB 14 2001

Disclaimer

This review may have been altered subsequent to the contractor's signatures above.

Oak Ridge National Laboratory Managed and Operated by UT-Battelle, LLC., for the U.S. Department of Energy under Contract No. DE-AC05-00OR22725.

CYHALOFOP BUTYL **Acute Oral Study [870.1100 (§81-1)]**

EPA Reviewer: John Whalan
Registration Action Branch 2 (7509C)
EPA Work Assignment Manager: SanYvette Williams, D.V.M.
Registration Action Branch 2 (7509C)
TXR No.: 0050348

John Whalan, Date 3-27-01
[signature], Date 5/31/01

DATA EVALUATION RECORD

STUDY TYPE: Acute Oral Toxicity - Rat [OPPTS 870.1100 (§81-1)]

DP BARCODE: D268553
P.C. CODE: 082583

SUBMISSION CODE: S
TOX. CHEM. NO.: None

TEST MATERIAL (PURITY): XRD-537 (97.1%)

SYNONYMS: (R)-(+)-N-butyl 2-[4-(2-fluoro- 4-cyanophenoxy)phenoxy]propionate

CITATION: Kosaka, T. et al. (1992) XRD-537: Acute oral toxicity study in rats. Kodaira Laboratories, the Institute of Environmental Toxicology, Suzuki-cho 2-772, Kodaira-shi, Tokyo 187, Japan. Laboratory study ID GHF-R 260, January 13, 1992. MRID 45000237. Unpublished.

SPONSOR: DowElanco Japan Limited, 2-1, Shibaura 1-chome, Minato-ku, Tokyo 105, Japan

EXECUTIVE SUMMARY: In an acute oral toxicity study (MRID 45000237) five male and five female fasted young adult SD rats (Crj:CD) were given a single oral 5000 mg/kg dose of XRD-537 (97.1% a.i., Lot No. AGR-295713) in aqueous 1% Tween 80 and observed for 14 days.

No rats died and no abnormal clinical signs were detected during the study. All rats gained weight during the study. No gross abnormalities were noted at necropsy.

The oral LD_{50} for males, females, and combined was > 5000 mg/kg (Limit Test).

XRD-537 is in TOXICITY CATEGORY IV based on the LD_{50}.

This acute oral study is classified as **Acceptable/Guideline** and satisfies the subdivision F guideline requirements for an acute oral study [870.1100 (§81-1)] in the rat.

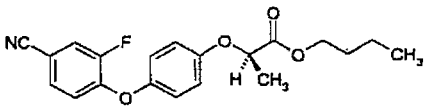
COMPLIANCE: Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: XRD-537

Description: off-white powder
Lot/Batch #: AGR-295713
Purity: 97.1% a.i.
CAS No.: 122008-85-9 for cyhalofop butyl ester
Structure:



2. Vehicle and/or positive control

1% Tween 80 aqueous solution

3. Test animals

Species: rat
Strain: SD (Crj:CD)
Age and/or weight at dosing: 6 weeks; males: 157-170 g, females: 131-144 g
Source: Charles River Japan, Inc., Shimofurusawa, Atsugi-shi, Kanagawa, Japan
Acclimation period: 7 days
Diet: Certified Pellet Diet MF, Oriental Yeast Co., Ltd., Azusawa, Itabashi-ku, Tokyo, *ad libitum*
Water: tap water, *ad libitum*
Housing: five/sex/wire-mesh stainless steel cage
Environmental conditions:
Temperature: 22.9-24.2 °C
Humidity: 54-61%
Air changes: 12/hour
Photoperiod: 12 hour light/dark

B. STUDY DESIGN AND METHODS

1. In life dates

Start: November 7, 1991; end: November 21, 1991

2. Animal assignment and treatment

The study was conducted as a limit test. Following an overnight fast, five rats/sex were given a single 5000 mg/kg dose of the test material in aqueous 1 % Tween 80 by gavage. The animals were observed for clinical signs of toxicity at 1, 3, and 6 hours post dosing and at least once daily thereafter for 14 days. They were weighed prior to dosing and on study days 7 and 14. All rats were sacrificed and necropsied.

3. Statistics

Calculation of the oral LD_{50} was not required.

II. RESULTS AND DISCUSSION

A. MORTALITY

None of the rats died as a result of XRD-537 toxicity.

The oral LD_{50} for males, females, and combined was > 5000 mg/kg. This places XRD-537 in TOXICITY CATEGORY IV.

B. CLINICAL OBSERVATIONS

No abnormal clinical signs were detected during the study.

C. BODY WEIGHT

All rats gained weight during the study.

D. NECROPSY

No gross abnormalities were noted.

E. DEFICIENCIES

None

DATA EVALUATION RECORD

CYHALOFOP BUTYL
(XRD-537)

STUDY TYPE: ACUTE ORAL TOXICITY - MOUSE [870.1100 (§81-1)]
MRID 45000238

Prepared for
Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
1921 Jefferson Davis Highway
Arlington, VA 22202

Prepared by
Chemical Hazard Evaluation Group
Toxicology and Risk Analysis Section
Life Sciences Division
Oak Ridge National Laboratory
Oak Ridge, TN 37831
Task Order No. 01-81C

Primary Reviewer:
Susan Chang, M.S.

Signature: Susan Chang
Date: FEB 14 2001

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Signature: H.T. Borges
Date: FEB 14 2001

Robert H. Ross, M.S., Group Leader

Signature: Robert H. Ross
Date: FEB 14 2001

Quality Assurance:
Lee Ann Wilson, M.A.

Signature: L. A. Wilson
Date: FEB 14 2001

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Oak Ridge National Laboratory Managed and Operated by UT-Battelle, LLC., for the U.S. Department of Energy under Contract No. DE-AC05-00OR22725.

CYHALOFOP BUTYL **Acute Oral Study [870.1100 (81-1)]**

EPA Reviewer: John Whalan
Registration Action Branch 2 (7509C)
EPA Work Assignment Manager: SanYvette Williams, D.V.M.
Registration Action Branch 2 (7509C)
TXR No.: 0050348

John Whalan, Date 3-27-01
, Date 5/31/01

DATA EVALUATION RECORD

STUDY TYPE: Acute Oral Toxicity - Mouse [OPPTS 870.1100 (§81-1)]

DP BARCODE: D268553
P.C. CODE: 082583

SUBMISSION CODE: S
TOX. CHEM. NO.: None

TEST MATERIAL (PURITY): XRD-537 (97.1%)

SYNONYMS: (R)-(+)-N-butyl 2-[4-(2-fluoro- 4-cyanophenoxy)phenoxy]propionate

CITATION: Kosaka, T. et al. (1992) XRD-537: Acute oral toxicity study in mice. Kodaira Laboratories, the Institute of Environmental Toxicology, Suzuki-cho 2-772, Kodaira-shi, Tokyo 187, Japan. Laboratory study ID GHF-R 258, January 13, 1992. MRID 45000238. Unpublished.

SPONSOR: DowElanco Japan Limited, 2-1, Shibaura 1-chome, Minato-ku, Tokyo 105, Japan

EXECUTIVE SUMMARY: In an acute oral toxicity study (MRID 45000238) five male and five female fasted young adult ICR mice (Crj:CD-1) were given a single oral 5000 mg/kg dose of XRD-537 (97.1% a.i., Lot No. AGR-295713) in aqueous 1% Tween 80 and observed for 14 days.

No mice died and no abnormal clinical signs were detected during the study. All mice gained weight during the study. No gross abnormalities were noted at necropsy.

The oral LD_{50} for males, females, and combined was > 5000 mg/kg (Limit Test).

XRD-537 is in TOXICITY CATEGORY IV based on the LD_{50}.

This acute oral study is classified as **Acceptable/Guideline** and satisfies the subdivision F guideline requirements for an acute oral study [870.1100 (§81-1)] in the mouse.

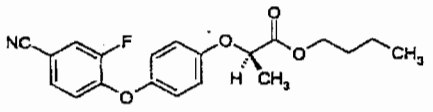
COMPLIANCE: Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: XRD-537

Description: off-white powder
Lot/Batch #: AGR-295713
Purity: 97.1% a.i.
CAS No.: 122008-85-9 for cyhalofop butyl ester
Structure:



2. Vehicle and/or positive control

1% Tween 80 aqueous solution

3. Test animals

Species: mouse
Strain: ICR (Crj:CD-1)
Age and/or weight at dosing: 6 weeks; males: 30.8-35.2 g, females: 21.7-25.3 g
Source: Charles River Japan, Inc., Shimofurusawa, Atsugi-shi, Kanagawa, Japan
Acclimation period: 6 days
Diet: Certified Pellet Diet MF, Oriental Yeast Co., Ltd., Azusawa, Itabashi-ku, Tokyo, *ad libitum*
Water: tap water, *ad libitum*
Housing: five/sex/aluminum cage with wire-mesh floor
Environmental conditions:
Temperature: 21.5-23.9°C
Humidity: 52-60%
Air changes: 12/hour
Photoperiod: 12 hour light/dark

B. STUDY DESIGN AND METHODS

1. In life dates

Start: November 7, 1991; end: November 21, 1991

2. Animal assignment and treatment

The study was conducted as a limit test. Following a two hour fast, five mice/sex were given a single 5000 mg/kg dose of the test material in aqueous 1% Tween 80 by

gavage. The animals were observed for clinical signs of toxicity at 1, 3, and 6 hours post dosing and at least once daily thereafter for 14 days. They were weighed prior to dosing and on study days 7 and 14. All mice were sacrificed and necropsied.

3. Statistics

Calculation of the oral LD_{50} was not required.

II. RESULTS AND DISCUSSION

A. MORTALITY

None of the mice died as a result of XRD-537 toxicity.

The oral LD_{50} for males, females, and combined was > 5000 mg/kg. This places XRD-537 in TOXICITY CATEGORY IV.

B. CLINICAL OBSERVATIONS

No abnormal clinical signs were detected during the study.

C. BODY WEIGHT

All mice gained weight during the study.

D. NECROPSY

No gross abnormalities were noted.

E. DEFICIENCIES

None

DATA EVALUATION RECORD

CYHALOFOP BUTYL
(XRD-537)

STUDY TYPE: ACUTE DERMAL TOXICITY - RAT [870.1200 (§81-2)]
MRID 45000240

Prepared for
Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
1921 Jefferson Davis Highway
Arlington, VA 22202

Prepared by
Chemical Hazard Evaluation Group
Toxicology and Risk Analysis Section
Life Sciences Division
Oak Ridge National Laboratory
Oak Ridge, TN 37831
Task Order No. 01-81E

Primary Reviewer:
Susan Chang, M.S.
Signature: Ross Chang
Date: FEB 14 2001

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Date: FEB 14 2001

Robert H. Ross, M.S., Group Leader
Signature: Robert H. Ross
Date: FEB 14 2001

Quality Assurance:
Lee Ann Wilson, M.A.
Signature: L. A. Wilson
Date: FEB 14 2001

Disclaimer

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Oak Ridge National Laboratory Managed and Operated by UT-Battelle, LLC., for the U.S. Department of Energy under Contract No. DE-AC05-00OR22725.

CYHALOFOP BUTYL **Acute Dermal Study [870.1200 (§81-2)]**

EPA Reviewer: John Whalan, Date 3-27-01
Registration Action Branch 2 (7509C)
EPA Work Assignment Manager: SanYvette Williams, D.V.M., Date 5/31/01
Registration Action Branch 2 (7509C)
TXR No.: 0050348

DATA EVALUATION RECORD

STUDY TYPE: Acute Dermal Toxicity - Rat [OPPTS 870.1200 (§81-2)]

DP BARCODE: D268553
P.C. CODE: 082583

SUBMISSION CODE: S
TOX. CHEM. NO.: None

TEST MATERIAL (PURITY): XRD-537 (97.1%)

SYNONYMS: (R)-(+)-N-butyl 2-[4-(2-fluoro- 4-cyanophenoxy)phenoxy]propionate

CITATION: Kosaka, T. et al. (1992) XRD-537: Acute dermal toxicity study in rats. Kodaira Laboratories, the Institute of Environmental Toxicology, Suzuki-cho 2-772, Kodaira-shi, Tokyo 187, Japan. Laboratory study ID GHF-R 259, January 13, 1992. MRID 45000240. Unpublished.

SPONSOR: DowElanco Japan Limited, 2-1, Shibaura 1-chome, Minato-ku, Tokyo 105, Japan

EXECUTIVE SUMMARY: In an acute dermal toxicity study (MRID 45000240) an approximately 4x5 cm area on the back of five male and five female young adult SD rats (Crj:CD) was dermally exposed to 2000 mg/kg XRD-537 (97.1% a.i., Lot No. AGR-295713) in distilled water for 24 hours. A control group with five males and five females was treated with distilled water only. The animals were observed for 14 days.

No rats died and no abnormal clinical signs were detected during the study. All rats gained weight during the study. No gross abnormalities were noted at necropsy.

The dermal LD_{50} for males, females, and combined was > 2000 mg/kg.

XRD-537 is in TOXICITY CATEGORY III based on the LD_{50}.

This acute dermal study is classified as **Acceptable/Guideline** and satisfies the subdivision F guideline requirements for an acute dermal study [870.1200 (§81-2)] in the rat.

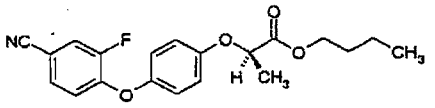
COMPLIANCE: Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: XRD-537

Description: off-white powder
Lot/Batch #: AGR-295713
Purity: 97.1% a.i.
CAS No.: 122008-85-9 for cyhalofop butyl ester
Structure:



2. Vehicle and/or positive control

Distilled water

3. Test animals

Species: rat
Strain: SD (Crj:CD)
Age and/or weight at dosing: 8 weeks; males: 308-353 g, females: 199-216 g
Source: Charles River Japan, Inc., Shimofurusawa, Atsugi-shi, Kanagawa, Japan
Acclimation period: 12 days
Diet: Certified Pellet Diet MF, Oriental Yeast Co., Ltd., Azusawa, Itabashi-ku, Tokyo, *ad libitum*
Water: tap water, *ad libitum*
Housing: individually in stainless steel cages
Environmental conditions:
Temperature: 22.8-24.2°C
Humidity: 54-61%
Air changes: 12/hour
Photoperiod: 12 hour light/dark

B. STUDY DESIGN AND METHODS

1. In life dates

Start: November 12, 1991; end: November 26, 1991

2. Animal assignment and treatment

Five male and five female rats were given a single 2000 mg/kg dose of XRD-537 applied to a 4x5 cm shaved skin site on the back. The application site was covered with a filter paper moistened with distilled water by a closed patch for 24 hours. The excess test material was washed with lukewarm water and neutral soap. Five control males and five control females were given 0.5 mL of distilled water with no test material. The animals were observed for clinical signs of toxicity 1, 3, and 6 hours after treatment and at least daily thereafter for 14 days. They were weighed prior to test material application, and on study days 7 and 14. All rats were sacrificed and necropsied.

3. Statistics

Calculation of the dermal LD_{50} was not required.

II. RESULTS AND DISCUSSION

A. MORTALITY

None of the rats died during the study.

The dermal LD_{50} for males, females, and combined was > 2000 mg/kg. This places XRD-537 in TOXICITY CATEGORY III.

B. CLINICAL OBSERVATIONS

No abnormal clinical signs were detected during the study.

C. BODY WEIGHT

All rats had normal body weight gains.

D. NECROPSY

No gross abnormalities were noted.

E. DEFICIENCIES

None

DATA EVALUATION RECORD

CYHALOFOP BUTYL
(DE-537 N-BUTYL ESTER)

STUDY TYPE: ACUTE DERMAL TOXICITY - RAT [870.1200 (§81-2)]
MRID 45000241, 45381901

Prepared for
Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
1921 Jefferson Davis Highway
Arlington, VA 22202

Prepared by
Chemical Hazard Evaluation Group
Toxicology and Risk Analysis Section
Life Sciences Division
Oak Ridge National Laboratory
Oak Ridge, TN 37831
Task Order No. 01-81F

Primary Reviewer:
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Signature: S. Chang
Date: FEB 14 2001

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Signature: H.T. Borges
Date: FEB 14 2001

Robert H. Ross, M.S., Group Leader

Signature: Robert H. Ross
Date: FEB 14 2001

Quality Assurance:
Lee Ann Wilson, M.A.

Signature: L. A. Wilson
Date: FEB 14 2001

Disclaimer

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Oak Ridge National Laboratory Managed and Operated by UT-Battelle, LLC., for the U.S. Department of Energy under Contract No. DE-AC05-00OR22725.

CYHALOFOP BUTYL **Acute Dermal Study [870.1200 (§81-2)]**

EPA Reviewer: John Whalan _____ Date 5-29-01
Registration Action Branch 2 (7509C)
EPA Work Assignment Manager: SanYvette Williams, D.V.M. _____, Date 5/31/01
Registration Action Branch 2 (7509C)
TXR No.: 0050348

DATA EVALUATION RECORD

STUDY TYPE: Acute Dermal Toxicity - Rat [OPPTS 870.1200 (§81-2)]

DP BARCODE: D268553
P.C. CODE: 082583

SUBMISSION CODE: S
TOX. CHEM. NO.: None

TEST MATERIAL (PURITY): DE-537 N-butyl ester (96.4%)

SYNONYMS: Not reported

CITATION: Stebbins, K.E. and K.J. Brooks (1998) DE-537 N-butyl ester: Acute dermal toxicity study in Fischer 344 rats. Health & Environmental Research Laboratories, The Dow Chemical Company, Midland, MI 48674. Laboratory project study ID 981083, July 6, 1998. MRID 45000241. Unpublished.

Stebbins, K.E. and K.J. Brooks (2001) Revised Report for DE-537 N-butyl ester: Acute dermal toxicity study in Fischer 344 rats. Health & Environmental Research Laboratories, The Dow Chemical Company, Midland, MI 48674. Laboratory project study ID 981083, March 15, 2001. MRID 45381901. Unpublished.

SPONSOR: Dow AgroSciences (DAS) LLC, 9330 Zionsville Road, Indianapolis, IN 46268

EXECUTIVE SUMMARY: In an acute dermal toxicity study (MRID 45381901) the back of five male and five female young adult Fischer 344 rats was dermally exposed to 5000 mg/kg DE-537 N-butyl ester (96.4% a.i., Lot No. DECO-26-42T, moistened with distilled water to form a paste) for 24 hours. The animals were observed for 14 days.

No rats died during the study. Two males had chromodacryorrhea on day 2 with recovery by day 3. All other rats were normal and no rats had dermal irritation during the study. All rats lost weight on day 2 and one male and five females lost weight through day 8. Four males gained weight by day 8 and all rats gained weight by the end of the study. No visible lesions were noted at necropsy.

The dermal LD_{50} for males, females, and combined was > 5000 mg/kg.

DE-537 N-butyl ester is in TOXICITY CATEGORY IV based on the LD_{50}.

This acute dermal study is classified as **Acceptable/Guideline** and satisfies the subdivision F guideline requirements for an acute dermal study [870.1200 (§81-2)] in the rat.

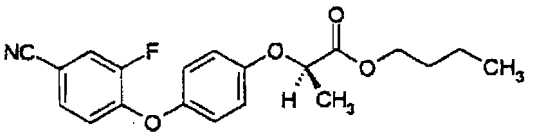
COMPLIANCE: Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: DE-537 N-butyl ester

   Description: off-white powder
   Lot/Batch #: DECO-26-42T; Reference No. AGR295713
   Purity: 96.4% a.i.
   CAS No.: 122008-85-9 for cyhalofop butyl ester
   Structure:



2. Vehicle and/or positive control

   None

3. Test animals

   Species: Rat
   Strain: Fischer 344
   Age and/or weight at dosing: approximately 10 weeks; males: 224.6-242.8 g, females: 143.3-146.3 g
   Source: Charles River Laboratories Inc., Raleigh, NC
   Acclimation period: not reported
   Diet: Certified Lab Diet No. 5002 (PMI Feeds, St. Louis, MO), *ad libitum*
   Water: tap water, *ad libitum*
   Housing: not reported
   Environmental conditions:
   Temperature: 22±3 °C
   Humidity: 40-70%
   Air changes: 12-15/hour
   Photoperiod: 12 hour light/dark

B. STUDY DESIGN AND METHODS

1. In life dates

Start: May 19, 1998; end: June 2, 1998

2. Animal assignment and treatment

Five male and five female rats were given a single 5000 mg/kg dose of DE-537 N-butyl ester applied to a clipped area on the dorsal trunk. The test article was moistened with 0.9 mL of distilled water to form a paste. The application site (approximately 10% of the surface area of the rats) was covered with a gauze patch and secured with elastic tape for 24 hours. The excess test material was removed with a disposable towel moistened with water. The animals were observed for clinical signs of toxicity frequently after treatment and at least once each work day thereafter for 14 days. They were weighed prior to test material application, and on study days 2, 8, and 15. All rats were sacrificed and necropsied.

3. Statistics

Calculation of the dermal LD_{50} was not required.

II. RESULTS AND DISCUSSION

A. MORTALITY

None of the rats died during the study.

The dermal LD_{50} for males, females, and combined was > 5000 mg/kg. This places DE-537 N-butyl ester in TOXICITY CATEGORY IV.

B. CLINICAL OBSERVATIONS

Two males had chromodacryorrhea on day 2 with recovery by day 3. All other rats were normal and no rats had dermal irritation during the study.

C. BODY WEIGHT

All rats lost weight on day 2 and one male and five females lost weight through day 8. Four males gained weight by day 8. All rats gained weight by the end of the study.

D. NECROPSY

No visible lesions were noted.

E. DEFICIENCIES

None

NOTE: The revised study report was submitted to correct an omission in the original report regarding the moistening of the test article to form a paste.

DATA EVALUATION RECORD

CYHALOFOP BUTYL
(XRD-537 BE)

STUDY TYPE: ACUTE INHALATION TOXICITY - RAT [870.1300 (§81-3)]
MRID 45000401

Prepared for
Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
1921 Jefferson Davis Highway
Arlington, VA 22202

Prepared by
Chemical Hazard Evaluation Group
Toxicology and Risk Analysis Section
Life Sciences Division
Oak Ridge National Laboratory
Oak Ridge, TN 37831
Task Order No. 01-81H

Primary Reviewer:
Susan Chang, M.S.

Signature: Susan Chang
Date: FEB 14 2001

Secondary Reviewers:
H. Tim Borges, M.T.(A.S.C.P.), Ph.D., D.A.B.T.

Signature: H.T. Borges
Date: FEB 14 2001

Robert H. Ross, M.S., Group Leader

Signature: Robert H. Ross
Date: FEB 14 2001

Quality Assurance:
Lee Ann Wilson, M.A.

Signature: L. A. Wilson
Date: FEB 14 2001

Disclaimer

This review may have been altered subsequent to the contractor's signatures above.

Oak Ridge National Laboratory Managed and Operated by UT-Battelle, LLC., for the U.S. Department of Energy under Contract No. DE-AC05-00OR22725.

CYHALOFOP BUTYL **Acute Inhalation Study [870.1300 (§81-3)]**

EPA Reviewer: John Whalan, Date 3-28-01
Registration Action Branch 2 (7509C)
EPA Work Assignment Manager: SanYvette Williams, D.V.M., Date 5/31/01
Registration Action Branch 2 (7509C)
TXR No.: 0050348

DATA EVALUATION RECORD

STUDY TYPE: Acute Inhalation Toxicity - Rat [OPPTS 870.1300 (§81-3)]

DP BARCODE: D268553
P.C. CODE: 082583

SUBMISSION CODE: S
TOX. CHEM. NO.: None

TEST MATERIAL (PURITY): XRD-537 BE (96.0%)

SYNONYMS: (R)-(+)-N-butyl 2-[4-(2-fluoro- 4-cyanophenoxy)phenoxy]propanoate

CITATION: Ebino, K., et al. (1994) XRD-537 BE: Acute inhalation toxicity study in rats. Mitsukaido Laboratories, The Institute of Environmental Toxicology, 4321, Uchimoriya-cho, Mitsukaido-shi, Ibaraki 303, Japan. Laboratory study ID IET 90-0179, January 17, 1994. MRID 45000401. Unpublished.

SPONSOR: Dow Chemical Japan Ltd., Seavans North, 2-1, Shibaura 1-chome, Minato-ku, Tokyo 105, Japan.

EXECUTIVE SUMMARY: In an acute inhalation toxicity study (MRID 45000401), five male and five female young adult Fischer 344 rats were exposed whole body to XRD-537 BE (96.0%, Lot No. AGR 295713) mixed with "white carbon" ($SiO_2 \cdot nH_2O$) for 4 hours at an analytical concentration of 5.63 mg/L. In addition, five males and five females were exposed to the white carbon vehicle using the same procedure as for the test animals. The mass median aerodynamic diameter (MMAD) for XRD-537 BE was 5.2 μm with a geometric standard deviation of 2.4. Approximately 29% of the particles were < 3.3 μm. The animals were observed for 14 days.

No rats died during the study. Visual observations were impeded during the exposure due to the opacity of the chamber atmosphere. Bradypnea was noted in all test rats with recovery within two hours following exposure. Abnormal respiratory sounds were noted from all test rats after exposure with recovery by day 1. Reddish adhesive materials in the nasorostral and periocular regions were noted from all test rats after exposure with recovery by day 2. Two control rats had reddish adhesive materials in the nasorostral region after exposure with recovery by two hours. All rats had normal body weight gains. No gross abnormalities were noted at necropsy.

The LC_{50} for males, females, and combined sexes was > 5.63 mg/L.

XRD-537 BE is in TOXICITY CATEGORY IV based on the LC_{50}.

This acute inhalation study is classified as **Acceptable/Guideline** and satisfies the subdivision F guideline requirements for an acute inhalation study [870.1300 (§81-3)] in the rat. Although the MMAD in this study (5.2 μm) slightly exceeds the recommended MMAD range (1-4 μm), the fact that no rats died at an analytical concentration nearly three times the limit concentration (2 mg/L) is sufficient to justify a Toxicity Category of IV.

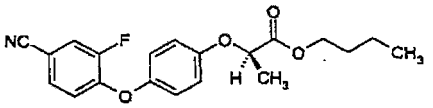
COMPLIANCE: Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: XRD-537 BE; pulverized mixture of 80 parts of XRD-537 BE technical and 20 parts w/w of the vehicle

   Description: sticky off-white powder; becomes fine after addition of vehicle ($SiO_2 \cdot nH_2O$)
   Lot/Batch #: AGR 295713
   Purity: 96.0%.
   CAS No.: 122008-85-9 for cyhalofop butyl ester



2. Vehicle and/or positive control

   "White carbon" ($SiO_2 \cdot nH_2O$)

3. Test animals

   Species: rat
   Strain: Fischer 344/DuCrj
   Age and weight at dosing: 8 weeks; males: 205-225 g, females: 132-150 g
   Source: Atsugi Breeding Center, Charles River Japan, Inc., Kanagawa, Japan
   Acclimation period: 9-10 days
   Diet: certified pelletized diet MF (Lot No. 930309, Oriental Yeast Co., Ltd., Tokyo, Japan), *ad libitum*
   Water: sterilized water, *ad libitum*
   Housing: 5/sex/stainless steel wire cage placed in the isolated-ventilation racks
   Environmental conditions:
   Temperature: 20.6-25.4°C
   Humidity: 50-79%
   Air changes: 10/hour
   Photoperiod: 12 hour light/dark

B. STUDY DESIGN AND METHODS

1. In life dates

Start: June 17, 1993; end: July 3, 1993

2. Exposure conditions

Temperature and humidity were measured continuously during the 4 hour exposure.

3. Animal assignment and treatment

Animals were assigned to the test groups noted in Table 1. Five male and five female rats were dynamically exposed to XRD-537 BE mixed with "white carbon" ($SiO_2 \cdot nH_2O$) by whole body exposure for four hours. Because the test article is a sticky powder, it was necessary to add white carbon to permit pulverization to a finer particle size in a jet air mill. Five males and five females were exposed to the white carbon vehicle.

The rats were observed two hours after the initiation of exposure, immediately after the termination of exposure, and two hours after the termination of exposure, and at least once daily thereafter for 14 days. They were weighed prior to test material exposure and on days 7 and 14. All rats were sacrificed and necropsied.

TABLE 1. Concentrations, exposure conditions, mortality/animals treated

Nominal Conc. (mg/L)	Anal. Conc. (mg/L)	MMAD (μm)	GSD	Particles <3.3 μm (%)	Temp. (°C)	Humidity (%)	Mortality		
							Male	Female	Combined
0 [a]	-	2.7	3.0	~50	22-26	46-60	0/5	0/5	0/10
19.98	5.63 [b]	5.2	2.4	~29	25-26	45-55	0/5	0/5	0/10

Data were taken from pp. 15, 16, and 28, MRID 45000401.

[a] White carbon ($SiO_2 \cdot nH_2O$) vehicle control - Nominal concentration of white carbon = 6.60 mg/L

[b] This is the analytical atmospheric concentration of XRD-537 BE Technical. The analytical atmospheric concentration of test material was 7.44 mg/L and white carbon vehicle was 1.80 mg/L.

4. Generation of the test atmosphere and description of the chamber

The whole body exposure chamber volume was 380 L, and the average total airflow was 100 liters/min. The exposure atmosphere was generated by using a turntable-type dust feeder with filtered compressed air. Additional diluent air was supplied directly to the exposure chamber from filtered room air. Time to equilibrium was not reported.

Test atmosphere concentration - Gravimetric samples were collected three times per hour from the chamber sampling port during exposure using glass fiber filters. XRD-537 BE collected on the filter was extracted with acetonitrile. High-

performance liquid chromatography was used to determine the concentration of XRD-537 BE in the exposure chamber. The total amount of test material supplied to the dust feeder was multiplied by 0.786[1] and divided by the total air volume delivered to the exposure chamber to obtain the nominal concentration of XRD-537 BE technical in the exposure chamber. The mean results are in Table 1 above.

Particle size determination - Particle size distribution of each exposure concentration was determined at one and three hours during exposure using an Andersen type cascade impactor. The test material concentration collected by each stage was determined gravimetrically. The aerodynamic mass median diameter (MMAD) and geometric standard deviation (GSD) were determined by plotting on a logarithmic-probability paper. Results are in Table 1 above.

5. Statistics

Calculation of the inhalation LC_{50} was not required.

II. RESULTS AND DISCUSSION

A. MORTALITY

No rats died during the study.

The LC_{50} for males, females, and combined was > 5.63 mg/L.

B. CLINICAL OBSERVATIONS

Due to dust in the chamber, observation was not possible during exposure. Bradypnea was noted from all test rats with recovery by two hours after exposure. Abnormal respiratory sounds were noted from all test rats after exposure with recovery by day 1. Reddish adhesive materials in the nasorostral and periocular regions were noted from all test rats after exposure with recovery by day 2. Two control rats had reddish adhesive materials in the nasorostral region after exposure with recovery by two hours.

C. BODY WEIGHT

All rats had normal body weight gains.

D. NECROPSY

No gross abnormalities were noted.

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[1] The concentration of XRD-537 BE technical in the test material was calculated to be 78.6% based on the analytical concentration of XRD-537 BE (75.5%) in the test material and the purity of XRD-537 BE technical (96.0%).

E. DEFICIENCIES

The aerosol MMAD exceeded the recommended range of 1-4 μm.

DATA EVALUATION RECORD

CYHALOFOP BUTYL
(XRD-537 BE)

STUDY TYPE: PRIMARY EYE IRRITATION - RABBIT [870.2400 (§81-4)]
MRID 45000403

Prepared for
Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
1921 Jefferson Davis Highway
Arlington, VA 22202

Prepared by
Chemical Hazard Evaluation Group
Toxicology and Risk Analysis Section
Life Sciences Division
Oak Ridge National Laboratory
Oak Ridge, TN 37831
Task Order No. 01-81J

Primary Reviewer:
Susan Chang, M.S.

Signature: Susan Chang
Date: FEB 14 2001

Secondary Reviewers:
H. Tim Borges, M.T.(A.S.C.P.), Ph.D., D.A.B.T.

Signature: H T Borges
Date: FEB 14 2001

Robert H. Ross, M.S., Group Leader

Signature: Robert H. Ross
Date: FEB 14 2001

Quality Assurance:
Lee Ann Wilson, M.A.

Signature: L. A. Wilson
Date: FEB 14 2001

Disclaimer

This review may have been altered subsequent to the contractor's signatures above.

Oak Ridge National Laboratory Managed and Operated by UT-Battelle, LLC., for the U.S. Department of Energy under Contract No. DE-AC05-00OR22725.

CYHALOFOP BUTYL **Primary Eye Irritation Study [870.2400 (§81-4)]**

EPA Reviewer: John Whalan, Date 3-28-01
Registration Action Branch 2 (7509C)
EPA Work Assignment Manager: SanYvette Willaims, D.V.M., Date 5/31/01
Registration Action Branch 2 (7509C)
TXR No.: 0050348

DATA EVALUATION RECORD

STUDY TYPE: Primary Eye Irritation - Rabbit [OPPTS 870.2400 (§81-4)]

DP BARCODE: D268553
P.C. CODE: 082583

SUBMISSION CODE: S
TOX. CHEM. NO.: None

TEST MATERIAL (PURITY): XRD-537 BE (95.8%)

SYNONYMS: (R)-(+)-N-butyl 2-[4-(2-fluoro- 4-cyanophenoxy)phenoxy]propanoate

CITATION: Jones, J.R. (1993) XRD-537 BE: Primary eye irritation test in the rabbit. Safepharm Laboratories Limited, P.O. Box 45, Derby, DE1 2BT, United Kingdom. Laboratory study ID 413/9, October 20, 1993. MRID 45000403. Unpublished.

SPONSOR: DowElanco Japan Limited

EXECUTIVE SUMMARY: In a primary eye irritation study (MRID 45000403) 0.1 mL of XRD-537 BE (95.8%, Batch No. DECO-26-42T) was instilled into the right conjunctival sac of six adult male/female New Zealand white rabbits. The contralateral eye of all rabbits served as control. The eyes were scored for ocular irritation according to the Draize method 1, 24, 48, and 72 hours after instillation and the irritation classified according to the method of Kay and Calandra.

No corneal opacity was found in any rabbit. One rabbit had iritis one hour after test material instillation with recovery by 24 hours. The test material induced a positive conjunctival irritation (redness and chemosis) in another rabbit one hour after test material instillation that subsided by 24 hours with recovery by 48 hours. The maximum mean total score was 7.5 at one hour after test material instillation.

In this study, XRD-537 BE was minimally irritating and is in TOXICITY CATEGORY IV for primary eye irritation.

This study is classified as **Acceptable/Guideline** and satisfies the subdivision F guideline requirements for a primary eye irritation study [870.2400 (§81-4)] in the rabbit.

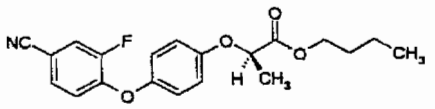
COMPLIANCE: Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: XRD-537 BE

Description: off-white powder
Lot/Batch #: DECO-26-42T
Purity: 95.8%.
CAS No.: 122008-85-9 for cyhalofop butyl ester
Structure:



2. Vehicle

None

3. Test animals

Species: rabbit
Strain: New Zealand White
Age and weight at dosing: approximately 13-17 weeks; males: 2.70-2.98 kg and females: 2.46-2.60 kg
Source: David Percival Ltd., Moston, Sandbach, Cheshire, U.K.
Acclimation period: at least 5 days
Diet: RABMA Rabbit Diet, Special Diet Services Ltd., Witham, Essex, U.K., *ad libitum*
Water: mains drinking water, *ad libitum*
Housing: individually in suspended metal cages
Environmental conditions:
Temperature: 21-23 °C
Humidity: 63-68%
Air changes: approximately 15/hour
Photoperiod: 12 hour light/dark

B. STUDY DESIGN AND METHODS

1. In life dates

Start: August 2, 1993; end: August 6, 1993

2. Animal assignment and treatment

The test material (0.1 mL ≅ 70 mg) was instilled into the right conjunctival sac of four male and two female rabbits and the eye lids held together for approximately 1 second. The contralateral eye of all rabbits served as control. The animals were scored for ocular irritation 1, 24, 48, and 72 hours after instillation according to the Draize method and the degree of irritation was classified according to the method of Kay and Calandra.

II. RESULTS AND DISCUSSION

A. No corneal opacity was found in any rabbit. One rabbit had iritis one hour after test material instillation with recovery by 24 hours. The test material induced a positive conjunctival irritation (redness and chemosis) in another rabbit one hour after test material instillation that subsided to score 1 (not positive) by 24 hours with recovery by 48 hours. The maximum mean total score was 7.5 at one hour after test material instillation.

This classifies the test material as minimally irritating. XRD-537 BE is in TOXICITY CATEGORY IV.

B. DEFICIENCIES

None

DATA EVALUATION RECORD

CYHALOFOP BUTYL
(XRD-537 BE)

STUDY TYPE: PRIMARY DERMAL IRRITATION - RABBIT [870.2500 (§81-5)]
MRID 45000405

Prepared for
Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
1921 Jefferson Davis Highway
Arlington, VA 22202

Prepared by
Chemical Hazard Evaluation Group
Toxicology and Risk Analysis Section
Life Sciences Division
Oak Ridge National Laboratory
Oak Ridge, TN 37831
Task Order No. 01-81M

Primary Reviewer:
Susan Chang, M.S.

Signature: Susan Chang
Date: FEB 14 2001

Secondary Reviewers:
H. Tim Borges, M.T.(A.S.C.P.), Ph.D., D.A.B.T.

Signature: H T Borges
Date: FEB 14 2001

Robert H. Ross, M.S., Group Leader

Signature: Robert H Ross
Date: FEB 14 2001

Quality Assurance:
Lee Ann Wilson, M.A.

Signature: L. A. Wilson
Date: FEB 14 2001

Disclaimer

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Oak Ridge National Laboratory Managed and Operated by UT-Battelle, LLC., for the U.S. Department of Energy under Contract No. DE-AC05-00OR22725.

CYHALOFOP BUTYL **Primary Dermal Irritation Study [870.2500 (§81-5)]**

EPA Reviewer: John Whalan
Registration Action Branch 2 (7509C)
EPA Work Assignment Manager: SanYvette Willaims, D.V.M.
Registration Action Branch 2 (7509C)
TXR. No.: 0050348

John Whalan, Date 3-29-01
, Date 5/31/05

DATA EVALUATION RECORD

STUDY TYPE: Primary Dermal Irritation - Rabbit [OPPTS 870.2500 (§81-5)]

DP BARCODE: D268553
P.C. CODE: 082583

SUBMISSION CODE: S
TOX. CHEM. NO.: None

TEST MATERIAL (PURITY): XRD-537 BE (95.8%)

SYNONYMS: (R)-(+)-N-butyl 2-[4-(2-fluoro- 4-cyanophenoxy)phenoxy]propanoate

CITATION: Jones, J.R. (1993) XRD-537 BE: Primary dermal irritation test in the rabbit. Safepharm Laboratories Limited, P.O. Box 45, Derby, DE1 2BT, United Kingdom. Laboratory study ID 413/8, October 20, 1993. MRID 45000405. Unpublished.

SPONSOR: DowElanco Japan Limited

EXECUTIVE SUMMARY: In a primary dermal irritation study (MRID 45000405) four male and two female adult New Zealand White rabbits were dermally exposed to 0.5 g XRD-537 BE (95.8%, Batch No. DECO-26-42T) moistened with 0.5 mL of distilled water for 4 hours on the dorsal/flank area. The animals were observed for 72 hours. Irritation was scored by the method of Draize.

Very slight erythema was noted on 1/6 rabbits one hour following patch removal that resolved by 24 hours. The primary dermal irritation index was 0.0.

In this study, XRD-537 BE was essentially nonirritating and is in TOXICITY CATEGORY IV for primary dermal irritation.

This study is classified as **Acceptable/Guideline** *and satisfies the subdivision F guideline* requirements for a primary dermal irritation study [870.2500 (§81-5)] in the rabbit.

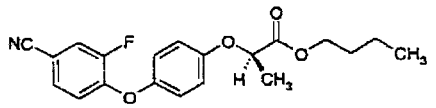
COMPLIANCE: Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: XRD-537 BE

Description: off-white powder
Lot/Batch #: DECO-26-42T
Purity: 95.8%.
CAS No.: 122008-85-9 for cyhalofop butyl ester



2. Vehicle

None

3. Test animals

Species: rabbit
Strain: New Zealand White
Age and weight at dosing: adult; approximately 13-17 weeks; males: 2.01-2.29 kg and females: 2.14-2.41 kg
Source: David Percival Ltd., Moston, Sandbach, Cheshire, U.K.
Acclimation period: at least 5 days
Diet: RABMA Rabbit Diet, Special Diet Services Ltd., Witham, Essex, U.K., *ad libitum*
Water: mains drinking water, *ad libitum*
Housing: individually in suspended metal cages
Environmental conditions:
Temperature: 19-23°C
Humidity: 59-67%
Air changes: approximately 15/hour
Photoperiod: 12 hour light/dark

B. STUDY DESIGN AND METHODS

1. In life dates

Start: July 22, 1993; end: July 25, 1993

2. Animal assignment and treatment

Four male and two female animals were given a single 0.5 g dose of XRD-537 BE moistened with 0.5 mL of distilled water and applied under a 2.5 cm x 2.5 cm gauze

patch to a clipped intact site on the dorsal/flank area. The patch was secured with surgical adhesive tape and the trunk was wrapped with an elastic corset. The semi-occlusive dressings were left in place for 4 hours, after which they were removed and the application sites wiped with cotton wool soaked in distilled water. The sites were scored for erythema and edema according to the Draize method 1, 24, 48, and 72 hours after patch removal.

II. RESULTS AND DISCUSSION

A. Very slight erythema was noted on 1/6 rabbits one hour following patch removal that resolved by 24 hours. The primary dermal irritation index was 0.0.

XRD-537 BE is essentially nonirritating and is in TOXICITY CATEGORY IV.

B. DEFICIENCIES

None

DATA EVALUATION RECORD

CYHALOFOP BUTYL
(DE-537 N-BUTYL ESTER)

STUDY TYPE: DERMAL SENSITIZATION - GUINEA PIG [870.2600 (§81-6)]
MRID 45000407

Prepared for
Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
1921 Jefferson Davis Highway
Arlington, VA 22202

Prepared by
Chemical Hazard Evaluation Group
Toxicology and Risk Analysis Section
Life Sciences Division
Oak Ridge National Laboratory
Oak Ridge, TN 37831
Task Order No. 01-81O

Primary Reviewer:
Susan Chang, M.S.

Signature: Susan Chang
Date: FEB 14 2001

Secondary Reviewers:
H. Tim Borges, M.T.(A.S.C.P.), Ph.D., D.A.B.T.

Signature: H.T. Borges
Date: FEB 14 2001

Robert H. Ross, M.S., Group Leader

Signature: Robert H. Ross
Date: FEB 14 2001

Quality Assurance:
Lee Ann Wilson, M.A.

Signature: L. A. Wilson
Date: FEB 14 2001

Disclaimer

This review may have been altered subsequent to the contractor's signatures above.

Oak Ridge National Laboratory Managed and Operated by UT-Battelle, LLC., for the U.S. Department of Energy under Contract No. DE-AC05-00OR22725.

CYHALOFOP BUTYL **Dermal Sensitization Study [870.2600 (§81-6)]**

EPA Reviewer: John Whalan
Registration Action Branch 2 (7509C)
EPA Work Assignment Manager: SanYvette Williams, D.V.M.
Registration Action Branch 2 (7509C)
TXR No.: 0050348

John Whalan, Date 3-29-01
Date 5/31/01

DATA EVALUATION RECORD

STUDY TYPE: Dermal Sensitization - Guinea Pig [OPPTS 870.2600 (§81-6)]

DP BARCODE: D268553
P.C. CODE: 082583

SUBMISSION CODE: S
TOX. CHEM. NO.: None

TEST MATERIAL (PURITY): DE-537 N-butyl ester (99.5%)

SYNONYMS: Not reported

CITATION: Johnson, I.R. (1996) DE-537 N-butyl ester: Delayed contact hypersensitivity study in the guinea pig. Huntingdon Life Sciences Ltd., Eye, Suffolk IP23 7PX, England. Laboratory study ID GHE-T-591, January 11, 1996. MRID 45000407. Unpublished.

SPONSOR: Dow AgroSciences LLC

EXECUTIVE SUMMARY: In a dermal sensitization study (MRID 45000407) with DE-537 N-butyl ester (99.5% w/w, Batch No. AGR 276542), 15 young adult male Dunkin-Hartley guinea pigs were tested using the method of Magnusson-Kligman Maximization.

All sites on the test animals exhibited slight to moderate erythema with or without discoloration or pallor after intradermal injection. With the exception of two control animals treated with propylene glycol that had no response, the other sites on the control animals had slight to moderate erythema with or without pallor after intradermal injection. After topical induction, the test animals developed exfoliation, while the control animals showed no response. Slight erythema with or without exfoliation was noted on 1/10 test animals and 1/5 control animals challenged with 50% w/v test material in propylene glycol. No dermal irritation was noted on any other animal after challenge. The study report include a HCA positive control study which was carried out within six months of the current study. The results were appropriate.

In this study, DE-537 N-butyl ester was not a dermal sensitizer.

This study is classified as **Acceptable/Guideline** and satisfies the subdivision F guideline requirements for a dermal sensitization study [870.2600 (§81-6)] in the guinea pig.

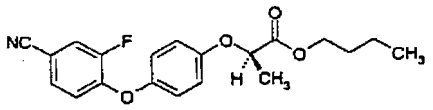
COMPLIANCE: Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: DE-537 N-butyl ester

Description: light buff lumpy crystalline powder
Lot/Batch #: AGR 276542
Purity: 99.5% w/w.
CAS No.: 122008-85-9 for cyhalofop butyl ester



2. Vehicle and positive control

Vehicle: propylene glycol or Freunds Complete Adjuvant; positive control: hexyl cinnamic aldehyde (HCA) (historical data)

3. Test animals

Species: guinea pig
Strain: Dunkin-Hartley
Age and weight at start of treatment: 6-8 weeks; body weight not reported
Source: David Hall, Darley Oaks, Newchurch, Burton on Trent, Staffordshire, England
Acclimation period: 6-16 days
Diet: pelleted guinea-pig diet (Guinea-pig F.D.1, Special Diets Services Limited, Witham, Essex, England), *ad libitum*
Water: tap water, *ad libitum*
Housing: individually in suspended stainless steel cages with mesh floors
Environmental conditions:
Temperature: 15-23°C
Humidity: 40-70%
Air changes: at least 10/hour
Photoperiod: 12 hour light/dark

B. STUDY DESIGN AND METHODS

1. In life dates

Start: August 22, 1995 (primary irritation screen); end: September 23, 1995

2. Animal assignment and treatment

The animals were induced and challenged according to the method of Magnusson-Kligman Maximization. Three pairs of intradermal injections (0.1 mL/site) were made into a 4 x 4 cm clipped area of dorsal skin on the scapular region of the guinea pigs (10 test and 5 control) on day 1. The injectables were Freund's complete adjuvant (diluted with equal volume of purified water), 1% w/v test material in propylene glycol, and 1% w/v test material in a 1:1 emulsion of Freund's complete adjuvant with purified water. On day 7, 0.5 mL of 10% w/v sodium lauryl sulfate in petroleum was applied to the clipped area of each animal. On day 8, the same scapular region was treated with 0.6 mL of 50% w/v test material in propylene glycol that was absorbed onto an absorbent patch and placed on the skin under occlusion for 48 hours. The naive control animals were treated similarly to the test animals with the exception that the vehicle was used in the place of test material for the intradermal injections and topical application. On day 21, the flanks of the test animals and the control animals were clipped. On day 22, the test animals and the control animals were topically challenged with 0.03 mL of 50% w/v test material in propylene glycol, 0.03 mL of 10% w/v test material in propylene glycol, and 0.03 mL of propylene glycol at naive sites on the left and right flanks for 24 hours. The sites were evaluated 24 and 48 hours post exposure.

II. RESULTS AND DISCUSSION

A. INDUCTION REACTIONS AND DURATION

All sites on the test animals exhibited slight to moderate erythema with or without discoloration or pallor at the dose site. With the exception of two control animals treated with propylene glycol that had no response, the other sites of the control animals had slight to moderate erythema with or without pallor at the dose site after intradermal induction. After topical induction, the test animals developed exfoliation, while the control animals showed no response.

B. CHALLENGE REACTIONS AND DURATION

Slight erythema with or without exfoliation was noted on 1/10 test animals and 1/5 control animals challenged with 50% w/v test material in propylene glycol. No dermal irritation was noted on any other animal.

DE-537 N-butyl ester was not a dermal sensitizer.

C. POSITIVE CONTROL

The study report included a HCA positive control study which was carried out within six months of the current study. The results were appropriate.

D. ADDITIONAL TESTING

It is the reviewer's opinion that the study was conducted in a manner suitable to detect the sensitization potential of the test material. No additional testing is needed.

E. DEFICIENCIES

The temperature of the animal room exceeded the required ranges (19-25°C). This would not affect the study results.

DATA EVALUATION REPORT

CYHALOFOP BUTYL
(XRD-537 NBU)

STUDY TYPE: SUBCHRONIC ORAL TOXICITY – RAT [OPPTS 870.3100a (82-1)]
MRID 45000413

Prepared for

Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
1921 Jefferson Davis Highway
Arlington, VA 22202

Prepared by

Chemical Hazard Evaluation Group
Toxicology and Risk Analysis Section
Life Sciences Division
Oak Ridge National Laboratory
Oak Ridge, TN 37831
Task Order No. 01-81R

Primary Reviewer: Carol S. Forsyth, Ph.D., D.A.B.T.	Signature: Date:	Carol S Forsyth JAN 23 2001
Secondary Reviewers: Sylvia S. Talmage, Ph.D., D.A.B.T.	Signature: Date:	Sylvia S. Talmage JAN 23 2001
Robert H. Ross, M.S., Group Leader	Signature: Date:	Robert H. Ross JAN 23 2001
Quality Assurance: Lee Ann Wilson, M.A.	Signature: Date:	L. A. Wilson JAN 23 2001

Disclaimer

This review may have been altered subsequent to the contractor's signatures above.

Oak Ridge National Laboratory, managed by UT-Battelle, LLC, for the U.S. Dept. of Energy under contract DE-AC05-00OR22725

CYHALOFOP BUTYL **Subchronic Oral Toxicity [OPPTS 870.3100 [§82-1a)]**

EPA Reviewer: J. Whalan, Date 5-8-01
Registration Action Branch 2 (7509)
EPA Work Assignment Manager: S. Williams-Foy, D.V.M., Date 5/31/01
Registration Action Branch 2 (7509)
TXR No.: 0050348

DATA EVALUATION RECORD

STUDY TYPE: Subchronic (4 and 13 Week) Oral Toxicity – Rat [OPPTS 870.3100 [§82-1a)]

DP BARCODE: D268553
P.C. CODE: 082583

SUBMISSION CODE: none
TOX. CHEM. NO.: none

TEST MATERIAL: Cyhalofop butyl (98.2% a.i.)

SYNONYMS: XRD-537 nBu; Propanoic acid: 2-(4-(4-cyano-2-fluorophenoxy)-phenoxy) n-butyl ester, (R(+))

CITATION: Corley, R.A., Haut, K.T., and Lomax, L.G. (1991) XRD-537 NBU: Four-week and 13-week dietary toxicity studies in Sprague-Dawley rats. The Toxicology Research Laboratory, Health and Environmental Sciences, The Dow Chemical Company, Midland, MI 48674. Laboratory Project ID: DR-0298-8876-003 and DR-0298-8876-003A, March 7, 1991. MRID 45000413, Unpublished.

SPONSOR: DowElanco, 9002 Purdue Road, Indianapolis, IN 46268-1189

EXECUTIVE SUMMARY: In a subchronic oral toxicity study (MRID 45000413), groups of Sprague-Dawley rats (10 rats/sex/group) were administered 0, 3 (males), 10 (females), 25 (males), 100, 400, or 800 (females) mg/kg/day of XRD-537 nBu (Batch No. AGR 276541; 98.2% a.i.) for at least 90 days. The test article was administered at dietary concentrations adjusted weekly to maintain a constant dose. A complete FOB, including handling and open field observations, was conducted during the week prior to necropsy on all surviving animals in the 90-day study; fore- and hind-limb grip strengths and motor activity were not measured. Satellite groups of 5 rats/sex were given 0, 25, 400 (males), 800 (females), or 1600 mg/kg/day for 4 weeks for determining organ weight and microscopic changes in potential target tissues.

All animals survived to scheduled sacrifice. During the 4-week study, perineal soiling was observed on days 16-28 on one male and one female at 1600 mg/kg/day. During the 13-week study, perineal soiling was observed after day 75 on 3/10 males and 3/10 females at 400 mg/kg/day, after day 72 on 1/10 females at 100 mg/kg/day, and as early as day 16 on 6/10 females at 800 mg/kg/day. This finding was not observed in controls or the lower dose groups. The only treatment-related abnormality noted during the FOB was perineal soiling observed on 1/10 males and 6/10 females in the high-dose groups.

During the 4-week study, body weights of the high-dose males and females were 70-81% and 71-85%, respectively, of the control group level. After 13 weeks of treatment, absolute body weights and body weight gains of the treated males were similar to those of the controls. Among females on the 13-week study, body weights of the high-dose group were significantly ($p \leq 0.05$) less than the controls on days 26, 40, and 54-89. Body weights of the high-dose females were 92% of the controls on day 26 and declined to 86% of the control level by termination. Overall weight gain by the high-dose females was 78% of the control value. Body weights and body weight gains by the lower dose female groups were similar to the control group throughout the study.

Food consumption data were not analyzed statistically and food efficiency was not calculated by the study authors. During the 4-week study, food consumption by the high-dose males was 88% of the control level for the first week and 92-107% thereafter. For the high-dose females, food consumption was reduced to 72% of the control level during the first week, but increased to 84% to 106% of the controls over the remainder of the study. In the 13-week study, food consumption by the treated males was similar to the controls throughout the study. However, food consumption by the high-dose females was 94% of the controls for the first week, declined to 76% of the controls for week 6, but returned to approximately the control levels by week 11. Food efficiency was not calculated.

Gross and microscopic findings indicative of liver enlargement due to enzyme induction were observed in both the 4- and 13-week studies. Absolute and relative liver weights were significantly ($p \leq 0.05$) increased in a dose-related manner and hepatocellular hypertrophy was observed in males at ≥25 mg/kg/day and in females at ≥100 mg/kg/day. After 13 weeks, alkaline phosphatase was increased ($p \leq 0.05$) in males and females administered ≥100 mg/kg/day. No ophthalmologic lesions were reported for any animal and urinalysis parameters were unaffected by treatment.

Therefore, the NOAEL for male rats is ≥400 mg/kg/day and the LOAEL for males is not identified. The LOAEL for female rats is 800 mg/kg/day based on perineal soiling and reduced body weights and body weight gain and the NOAEL for females is 400 mg/kg/day.

This study is classified as **Acceptable/Guideline** and satisfies the requirements for a subchronic oral toxicity study [OPPTS 870.3100 (§82-1a)] in rats.

COMPLIANCE: Signed and dated Quality Assurance, Data Confidentiality, Flagging, and Good Laboratory Practice Compliance statements were provided.

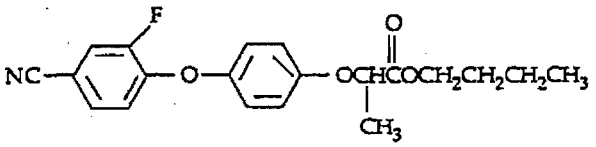
I. MATERIALS AND METHODS

A. MATERIALS

1. Test compound: XRD-537 nBu

   Description: solid
   CAS No.: not given

Batch No.: AGR 276541
Purity: 98.2% a.i.
Contaminants: none given
Structure:



2. Vehicle

Purina Certified Rodent Chow #5002 was used as the vehicle and negative control. The test article was dissolved in acetone to facilitate uniform dispersion in the diet. No positive control was used in this study.

3. Test animals

Species: Rat
Strain: Sprague-Dawley
Age and weight at study initiation: approx. 38 days: males, 126.2-155.0 g and females 98.4-155.8 g.
Source: Charles River Breeding Laboratory, Portage, MI
Housing: Animals were individually housed in stainless steel, wire-mesh cages suspended above cage boards.
Food: Purina Certified Rodent Chow #5002 was available *ad libitum.*
Water: Tap water was available *ad libitum.*
Environmental conditions:
Temperature: stated as adequate
Humidity: stated as adequate
Air changes: not given
Photoperiod: stated as adequate
Acclimation period: 10 days

B. STUDY DESIGN

1. In life dates

Start: not given; End: not given

2. Animal assignment

Animal assignment and dose selection are listed in Table 1. Animals were assigned to test groups using a computer-generated randomization scheme based on body weights. For the main study, 10 rats/sex/dose were administered test or control diets for 13 weeks. A satellite group of 5 rats/sex/dose were administered test or control diets for 4 weeks specifically for determining organ weight and microscopic changes in potential target tissues. Dietary concentrations were adjusted weekly based on the

most recent body weight and food consumption data in order to maintain constant dose levels.

TABLE 1. Study design

Test group	Dose (mg/kg/day)	No. of animals	
		Males	Females
4-week			
Control	0	5	5
Low-dose	25	5	5
Mid 1-dose	400	5	—
Mid 2-dose	800	—	5
High-dose	1600	5	5
13-week			
Control	0	10	10
Low-dose	3	10	—
Mid 1-dose	10	—	10
Mid 2-dose	25	10	—
Mid 3-dose	100	10	10
Mid 4-dose	400	10	10
High-dose	800	—	10

Data taken from Tables 1 and 2, pp. 31 and 32, respectively, MRID 45000413.

3. Rationale for dose selection

Dose levels in the current study were selected to facilitate comparisons with previous subchronic studies conducted with the methyl ester, XRD-537 ME, and to aid in selection of dose levels for subsequent chronic toxicity studies. In the rat, the liver was the primary target organ for XRD-537 ME following 4 or 13 weeks of treatment in previous studies.

4. Preparation and analysis of test diets

Test diets were prepared weekly during the study. The test article was dissolved in acetone to facilite uniform dispersion in the diet. The test diets were prepared by serially diluting the high-dose diet. The dietary concentrations were adjusted to maintain constant doses based on the most recent body weight and food consumption data. Concentration of the test article in each of the dietary levels was measured during test weeks 1, 5, 9, and 12 of the study. Homogeneity and stability data were included from previous reports by the sponsor, but the details were not included in the current report.

Results

Homogeneity analysis: Concentrations of the test article in samples taken from the top and bottom of a test diet varied by <10%.

Concentration: Absence of test article was confirmed in the control diets. Weekly concentrations of the test article in all diets were within 15% of nominal and the overall mean concentrations were within 10% of nominal.

Stability: The concentration of the test article in rodent feed was 91% of the initial measured concentration after 123 days. Details of storage conditions were not given.

Conclusion: These analyses confirm that the diets were homogeneously mixed and that the initial concentrations of the test article were acceptable.

5. Statistical analysis

Only means and standard deviations were reported for food consumption, red blood cell indices, and white cell counts. Body weight, organ weight, clinical chemistry data, appropriate hematology data, and urinary specific gravity were evaluated by Bartlett's test for equality of variances. Based on the outcome of Bartlett's test, exploratory data analysis was performed by a parametric or nonparametric analysis of variance (ANOVA), followed respectively by Dunnett's test or the Wilcoxon Rank-Sum test with a Bonferroni correction for multiple comparisons.

C. METHODS

1. Observations

Animals were observed once daily for clinical signs of toxicity and twice daily for mortality and moribundity. Detailed clinical examinations were conducted weekly on all animals.

2. Body weight

Body weights were recorded weekly during the study period.

3. Food consumption and food efficiency

Food consumption was measured weekly. Food efficiency was not calculated by the study authors.

4. Neurobehavioral evaluations

A complete FOB, including handling and open field observations, was conducted during the week prior to necropsy on all surviving animals in the 13-week study. Assessments were made of the eyes, movement/behavior, skin, fur, mucous

membranes, general condition, and feces and urine. Sensory responsiveness (touch, auditory, nociceptive) were made while the animal was in the open field. Fore- and hind-limb grip strengths and pupillary constriction were not measured. The treatment group of each animal was unknown to the observer.

5. Motor activity

Motor activity was not measured.

6. Ophthalmology

The eyes of all rats were examined prior to initiation of treatment (penlight illumination) and at week 4 or 13 scheduled necropsy (moist slide technique).

7. Clinical chemistry

Blood was collected from the orbital sinus of all rats in the 13-week study for hematology and clinical chemistry measurements. Rats were fasted prior to collection. Blood smears were prepared from all animals from which blood was collected. The CHECKED (X) parameters were evaluated:

a. Hematology

X		X	
X	Hematocrit (HCT)*	X	Leukocyte differential count*
X	Hemoglobin (HGB)*	X	Mean corpuscular HGB (MCH)[a]
X	Leukocyte count (WBC)*	X	Mean corpusc. HGB conc.(MCHC)[a]
X	Erythrocyte count (RBC)*	X	Mean corpusc. volume (MCV)[a]
X	Platelet count*		Reticulocyte count
	Blood clotting measurements*	X	Blood cell morphology
	(Activated thromboplastin time)		Red cell distribution width
	(Clotting time)		
	(Prothrombin time)		

*Required for subchronic studies based on OPPTS 870.3100 Guidelines.
[a]Males only.

b. Clinical chemistry

X	ELECTROLYTES	X	OTHER
X	Calcium*	X	Albumin*
X	Chloride*		Albumin/globulin ratio
	Magnesium	X	Blood creatinine*
X	Phosphorus*	X	Blood urea nitrogen*
X	Potassium*	X	Total Cholesterol
X	Sodium*	X	Globulins
		X	Glucose*
	ENZYMES	X	Total bilirubin
X	Alkaline phosphatase (ALK)	X	Total serum protein*
	Cholinesterase (ChE)	X	Triglycerides
X	Creatine phosphokinase		Serum protein electrophoresis
	Sorbitol dehydrogenase		
X	Alanine aminotransferase (also SGPT)*		
X	Aspartate aminotransferase (also SGOT)*		
	Gamma glutamyl transferase (GGT)		
	Glutamate dehydrogenase		

* Required for subchronic toxicity studies based on OPPTS 870.3100 Guidelines.

8. Urinalysis

Urine was collected by manual abdominal compression of all nonfasted surviving rats during the week prior to the 13-week scheduled sacrifice. The CHECKED (X) parameters were measured:

X		X	
X	Appearance	X	Glucose
	Volume	X	Ketones
X	Specific gravity	X	Bilirubin
X	pH	X	Blood
X	Sediment (microscopic)	X	Urobilinogen
X	Protein		Reducing substances

Urinalysis is not required for subchronic studies.

9. Sacrifice and pathology

All surviving rats were sacrificed after 4 or 13 weeks on study by decapitation while under methoxyflurane anesthesia and subjected to gross necropsy. Lungs were distended to an approximately normal inspiratory volume with neutral, phosphate-buffered 10% formalin. The following tissues (X) were collected from all animals and preserved in formalin. In addition, the (XX) tissues were weighed. All tissues from the control and high-dose animals and the liver, kidneys, and lungs from the animals in the lower dose groups of the 13 week study were examined microscopically. The liver and kidneys from all animals on the 4-week study were also examined microscopically. All gross lesions from any animal were examined microscopically.

X	DIGESTIVE SYSTEM	X	CARDIOVASC./HEMAT.	X	NEUROLOGIC
X	Oral tissues	X	Aorta*	XX	Brain*+
X	Tongue	XX	Heart*	X	Periph. nerve*
X	Salivary glands*	X	Bone marrow*	X	Spinal cord (3 levels)*
X	Esophagus*	X	Lymph nodes*	X	Pituitary
X	Stomach*	X	Spleen*	X	Eyes (optic n.)
X	Duodenum*	X	Thymus*		
X	Jejunum*				GLANDULAR
X	Ileum*		UROGENITAL	XX	Adrenal gland*
X	Cecum*	XX	Kidneys*+	X	Lacrimal gland
X	Colon*	X	Urinary bladder*	X	Auditory sebaceous gland
X	Rectum*	XX	Testes*+	X	Mammary gland*
XX	Liver*+	X	Epididymides*	X	Parathyroids*
X	Pancreas*	X	Prostate*	X	Thyroids*
		X	Seminal vesicle*	X	Coagulation glands
	RESPIRATORY	XX	Ovaries*		
X	Trachea*	X	Oviducts		OTHER
X	Lung*	X	Uterus*	X	Bone*
X	Nose (nasal turbinates)	X	Cervix	X	Skeletal muscle*
	Pharynx	X	Vagina	X	Skin*
X	Larynx			X	All gross lesions and masses*

* Required for subchronic toxicity studies based on OPPTS 870.3100 Guidelines.
+ Organ weight required in subchronic and chronic studies.

II. RESULTS

A. CLINICAL OBSERVATIONS AND MORTALITY

All animals survived to scheduled sacrifice. During the 4-week study, perineal soiling was observed on days 16-28 on one male and one female at 1600 mg/kg/day. During the 13-week study, perineal soiling was observed after day 75 on 3/10 males and 3/10 females at 400 mg/kg/day, after day 72 on 1/10 females at 100 mg/kg/day, and as early as day 16 on 6/10 females at 800 mg/kg/day. This finding was not observed in controls or the lower dose groups. No other treatment-related clinical signs of toxicity were observed.

B. BODY WEIGHTS AND BODY WEIGHT GAINS

During the 4-week study, body weights of the high-dose males and females were 70-81% and 71-85%, respectively, of the control group level.

Selected mean body weights and body weight gains during the 13-week study are listed in Table 2. Absolute body weights and body weight gains of the treated males were similar to those of the controls throughout the study. Among females, body weights of the high-dose group were significantly ($p \leq 0.05$) less than the controls on days 26, 40, and 54-89. Body weights of the high-dose females were 92% of the controls on day 26 and declined to 86% of the control level by termination. Overall weight gain by the high-

dose females was 78% of the control value. Body weights and body weight gains by the lower dose female groups were similar to the control group throughout the study.

TABLE 2: Selected body weights and body weight gains of male and female rats administered XRD-537 nBu in the diet for 13 weeks (g)

Males					
Day of study	**0 mg/kg/day**	**3 mg/kg/day**	**25 mg/kg/day**	**100 mg/kg/day**	**400 mg/kg/day**
-3	142.2	142.7	143.3	142.8	143.7
5	208.6	211.4	214.9	208.7	216.3
19	313.8	331.9	331.3	317.2	324.7
33	395.1	422.2	416.1	400.5	409.1
47	454.4	481.7	475.1	454.6	463.9
61	493.9	520.8	513.8	491.0	497.6
75	533.1	557.2	558.8	532.9	538.1
89	556.5	578.5	576.8	551.8	563.8
Wt. gain -3-5[a]	66.4	68.7	71.6	65.9	72.6
Wt. gain -3-89[a]	414.3	435.8	433.5	409.0	420.1
Females					
Day of study	**0 mg/kg/day**	**10 mg/kg/day**	**100 mg/kg/day**	**400 mg/kg/day**	**800 mg/kg/day**
-3	105.3	105.4	105.2	105.4	105.1
5	141.3	143.1	142.4	141.0	138.9
19	186.9	190.8	187.6	185.0	174.4
33	219.5	218.4	215.0	212.7	200.7
47	235.6	231.8	235.2	236.9	219.0
61	259.7	246.6	250.5	252.5	230.8* (89)
75	274.4	261.3	259.7	270.5	241.7* (88)
89	286.3	271.4	272.5	279.5	246.5* (86)
Wt. gain -3-5[a]	36.0	37.7	37.2	35.6	33.8
Wt. gain -3-89[a]	181.0	166.0	167.3	174.1	141.4 (78)

Data taken from Tables 21 and 22, pp. 60 and 61, respectively, MRID 45000413.
[a]Calculated by reviewer.
[b]Number in parentheses is percent of control; calculated by reviewer.
Significantly different from control: *$p \leq 0.05$.

C. FOOD CONSUMPTION AND COMPOUND INTAKE

1. Food consumption and food efficiency

Food consumption data were not analyzed statistically and food efficiency was not calculated by the study authors. During the 4-week study, food consumption by the

high-dose males was 88% of the control level for the first week and 92-107% thereafter. For the high-dose females, food consumption was reduced to 72% of the control level during the first week, but increased from 84% to 106% of the controls over the remainder of the study. Food consumption by the low- and mid-dose males and females was not affected by treatment for 4 weeks.

In the 13-week study, food consumption by the treated males was similar to the controls throughout the study. However, food consumption by the high-dose females was 94% of the controls for the first week, declined to 76% of the controls for week 6, but returned to approximately the control levels by week 11. Food consumption by the remaining treated female groups was similar to the control value throughout the study.

2. Compound intake

Dose levels are given in Table 1. The dietary concentrations were adjusted weekly to maintain constant doses based on the most recent body weight and food consumption data. The calculated doses (submitted by registrant in May, 2001) were 0, 3.34, 27.8, 1111, or 448 mg/kg/day in males, and 0, 11, 109, 444, or 872 mg/kg/day in females.

D. NEUROBEHAVIORAL EVALUATIONS

1. FOB

The only treatment-related abnormality noted during the FOB was perineal soiling observed on 1/10 males and 6/10 females in the high-dose groups.

2. Grip Strength

Fore- and hind-limb grip strengths were not measured.

3. Sensory Observations

No dose- or treatment-related changes in response to sensory stimuli were observed in males or females.

E. MOTOR ACTIVITY

Motor activity was not measured.

F. OPHTHALMOLOGY

No ophthalmologic lesions were reported for any animal.

G. CLINICAL PATHOLOGY

RBC counts, hemoglobin, and hematocrit were significantly ($p \leq 0.05$) reduced in the 100- and 400-mg/kg/day males to 91-94% and 88-91%, respectively, of the control values. However, the RBC indices of MCV, MCH, and MCHC for these groups were similar to the controls. No differences in any hematological parameter were noted in females.

Alkaline phosphatase levels were significantly ($p \leq 0.05$) increased in males and females at all doses ≥100 mg/kg/day as compared with their respective controls. In 100- and 400-mg/kg/day males, alkaline phosphatase was increased to 163% and 181%, respectively, of the control level. In the 100-, 400-, and 800-mg/kg/day females, alkaline phosphatase was increased to 154%, 151%, and 149%, respectively, of the control value. Serum albumin levels were significantly ($p \leq 0.05$) increased as compared with the control in the 400-mg/kg/day males (118%) and in the 400- and 800-mg/kg/day females (108% for both groups). Globulin levels were decreased ($p \leq 0.05$) in high-dose males and females to 81% and 88%, of the control levels. Other statistically significant differences in clinical chemistry parameters were not dose-related or were not of a magnitude as to be considered biologically significant.

H. URINALYSIS

No treatment-related differences were observed in urinalysis parameters.

I. SACRIFICE AND PATHOLOGY

1. Gross pathology

At the end of the 4-week study, an enlarged liver was observed grossly in 1, 4, and 5 males in the 25, 400, and 1600 mg/kg/day groups, respectively, and in 2 females in the 1600 mg/kg/day group.

After 13 weeks of treatment, an enlarged liver was observed grossly in 8/10 males and 1/10 females at 400 mg/kg/day and in 8/10 females at 800 mg/kg/day. Pale foci in the liver were also observed on 3/10 high-dose males, 1/10 females at 400 mg/kg/day, and 2/10 females at 800 mg/kg/day. Brown scabs or scales on the tail were noted for 5/10 and 9/10 females in the 400 and 800 mg/kg/day groups, respectively, and considered secondary to poor grooming.

2. Organ weights

Absolute and relative liver weights of males and females in the 4-week study were increased ($p \leq 0.05$) in a dose-related manner. For males in the 25-, 400-, and 1600-mg/kg/day groups, absolute and relative liver weights were 133% and 133%, 155% and 169%, and 151% and 206%, respectively of the controls. For females in the 25-, 800-, and 1600-mg/kg/day groups, absolute and relative liver weights were 106% and 107% (n.s.), 143% and 148%, and 150% and 206%, respectively of the controls. In

addition for the high-dose males and females, final body weights were decreased to 73% of the controls and relative kidney weights were increased to 125-130% ($p \leq 0.05$) of the control level.

Selected organ weight data for rats on the 13-week study are given in Table 3. Final body weights of the treated males were similar to the controls, however, final body weights of the high-dose females were significantly ($p \leq 0.05$) less than the controls. Absolute and relative liver weights were significantly ($p \leq 0.05$) increased in a dose-related manner in males at ≥25 mg/kg/day and in females at ≥100 mg/kg/day. For males in the 25, 100, and 400 mg/kg/day groups, absolute and relative liver weights were 110-115% (n.s.), 123-124%, and 163-164%, respectively, of the control values. For females in the 100, 400, and 800mg/kg/day groups liver weights were 114-118%, 131-134%, and 132-152%, respectively of the control levels. Absolute and/or relative kidney weights were also increased ($p \leq 0.05$; 114-122% of controls) in the 25- and 400-mg/kg/day males and the 800-mg/kg/day females. Other statistical differences in organ weights between the treated and control groups were not dose-related or were considered due to lower final body weights (females).

TABLE 2: Selected absolute and relative organ weights of male and female rats administered XRD-537 nBu in the diet for 13 weeks

Males					
Organ	**0 mg/kg/day**	**3 mg/kg/day**	**25 mg/kg/day**	**100 mg/kg/day**	**400 mg/kg/day**
Final body wt.	518.4	541.1	537.4	521.4	516.5
Liver Absolute (g) Relative (g/100g)	15.036 2.897	15.919 2.943	17.249 (115)[a] 3.201 (110)	18.710* (124) 3.576* (123)	24.546* (163) 4.742* (164)
Kidney Absolute (g) Relative (g/100g)	3.305 0.636	3.810 0.704	3.901* (118) 0.725* (114)	3.598 (109) 0.694 (109)	4.017* (122) 0.779* (122)
Females					
Organ	**0 mg/kg/day**	**10 mg/kg/day**	**100 mg/kg/day**	**400 mg/kg/day**	**800 mg/kg/day**
Final body wt.	260.0	249.9	251.6	255.6	227.3*
Liver Absolute (g) Relative (g/100g)	6.808 2.620	6.580 2.634	7.740* (114) 3.084* (118)	8.939* (131) 3.512* (134)	9.020* (132) 3.977* (152)
Kidney Absolute (g) Relative (g/100g)	1.775 0.685	1.684 0.675	1.701 0.680	1.830 0.719	1.803 0.794* (116)

Data taken from Tables 37 and 38, pp. 78 and 79, respectively, MRID 45000413.
[a]Number in parentheses is percent of control; calculated by reviewer.
Significantly different from control: *$p \leq 0.05$.

3. Microscopic pathology

At the end of the 4-week study, diffuse hepatocellular hypertrophy was observed microscopically in the livers from all treated males and from all mid- and high-dose females. Very slight or slight multifocal hepatocellular necrosis with accompanying inflammation was observed in all low- and mid-dose males, in 3 mid-dose females, and in 1 high-dose female.

Treatment-related microscopic lesions were limited to the liver in both males and females administered the test article for 13 weeks. Multifocal and/or diffuse hepatocellular hypertrophy was observed in 10/10 males in each of the 25, 100, and 400 mg/kg/day groups and in 10/10 females in each of the 100, 400, and 800 mg/kg/day groups. This lesion was not seen in the control or lowest dose groups of either sex. In addition, multifocal hepatocellular necrosis with accompanying inflammation was seen in 3/10 males at 100 mg/kg/day and in 2/10 males and 1/10 females at 400 mg/kg/day.

In females, diffuse hyperkeratosis of the epidermis was observed around the tails of 4/10 animals at 400 mg/kg/day and 9/10 animals at 800 mg/kg/day. The study author noted that for the 400-mg/kg/day group the number of gross lesions did not correspond to the number of microscopic lesions because the tail of one rat was inadvertently discarded.

III. DISCUSSION

A. DISCUSSION

No intercurrent deaths occurred during the study. Clinical signs of toxicity were limited to perineal soiling, however, the incidence rates were low with late onset in all groups except the 800-mg/kg/day females of the 13 week study. In this group, perineal soiling was observed as early as day 16 and on up to 60% of the animals. Whether this is an adverse effect is uncertain, but it may have been due to poor grooming secondary to the animals not feeling well. However, except for the reduced body weight gain by the 800-mg/kg/day females, no other findings were suggestive of poor health and perineal soiling was the only abnormality noted during the FOB.

Reduced body weights and body weight gains for the high-dose males and females on the 4 week study and the high-dose females on the 13 week study corresponded with reduced food consumption for these groups. The most pronounced effect on food consumption occurred early in both studies and may have been due to lack of palatability of the test article.

Slight reductions in some RBC parameters for the 100- and 400-mg/kg/day males of the 13 week study were not considered biologically significant or treatment-related. Similarly, the magnitude of the increases in albumin levels (400-mg/kg/day males) and the decreases in globulin levels (400-mg/kg/day males and 800-mg/kg/day females) were

not considered biologically significant. These differences in RBC and clinical chemistry endpoints were not accompanied by histological lesions. On the other hand, increases in alkaline phosphatase levels in males and females given ≥100 mg/kg/day were most likely due to compression of bile ducts as a result of liver enlargement rather than a direct effect of the test article.

The main effects of the test article were on the liver. Dose-related increases in the number of animals with a grossly enlarged liver and in absolute and relative liver weights occurred in both the 4–and 13-week studies. The increase in liver size was due to hepatocellular hypertrophy which was observed in males at ≥25 mg/kg/day and in females at ≥100 mg/kg/day. A common cause of hepatocellular hypertrophy is proliferation of the smooth endoplasmic reticulum due to enzyme induction as a result of exposure to a xenobiotic. The profile of effects seen here is similar to a phenobarbital-type induction and is considered an indication of exposure but not an adverse consequence.

The other liver lesion described in several animals was necrosis with inflammation. This lesion was only graded as very slight or slight, the incidences for males and females were not dose-related, and the lesion did not increase in either incidence or severity with a longer treatment duration. Focal necrosis is also a common finding in conjunction with hepatocellular hypertrophy. Therefore, this lesion is not considered an adverse effect of treatment.

Therefore, the NOAEL for male rats is ≥400 mg/kg/day and the LOAEL for males is not identified. The LOAEL for female rats is 800 mg/kg/day based on perineal soiling and reduced body weights and body weight gain and the NOAEL for females is 400 mg/kg/day.

This study is classified **Acceptable/Guideline** and satisfies the requirements for a subchronic oral toxicity study [OPPTS 870.3100 (§82-1a)] in rats.

B. STUDY DEFICIENCIES

No deficiencies were noted in the conduct of this study.

DATA EVALUATION RECORD

CYHALOFOP BUTYL
(XRD-537 BE)

STUDY TYPE: SUBCHRONIC ORAL TOXICITY-RAT
[OPPTS: 870.3100 (§82-1)]
MRID 45014705

Prepared for

Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
1921 Jefferson Davis Highway
Arlington, VA 22202

Prepared by

Chemical Hazard Evaluation Group
Toxicology and Risk Assessment Section
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Oak Ridge, TN 37831
Task Order No. 01-81Q

Primary Reviewer: Nancy B. Munro, Ph.D.	Signature: Date:	Robert H. Ross for N B Munro FEB 14 2001
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Disclaimer

This review may have been altered subsequent to the contractors' signatures above.

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CYHALOFOP BUTYL **Subchronic Oral Toxicity [OPPTS: 870.3100 (§82-1)]**

EPA Reviewer: J. Whalan Date: 5-8-01
Registration Action Branch 2 (7509C)
EPA Work Assignment Manager: S. Williams-Foy, D.V.M. Date: 5/31/01
Registration Action Branch 2 (7509C).
TXR No: 0050348

DATA EVALUATION RECORD

STUDY TYPE: Subchronic Oral Dietary Toxicity - Rat [OPPTS:870.3100 (§82-1)]

DP BARCODE: D268553 SUBMISSION CODE: S
P.C. CODE: 082583 CASE NO.: not given

TEST MATERIAL: Cyhalofop butyl

SYNONYMS: R-(+)-n-Butyl-2-(4-(2-fluoro-4-cyano-phenoxy) phenoxy) propanoate; XRD-537 BE; XDE-537 BE; XDE-537; XRD-537; XRD-537 nBu; XDE-537 nBu; XRD -537 n Butyl Ester; DEH-112

CITATION: Harada, T. et al. (1993) XRD-537 BE: 13-week oral subchronic toxicity study in rats. Mitsukaido Laboratories, The Institute of Environmental Toxicology, 4321, Uchimoriya-cho, Mitsukaido-shi, Ibaraki 303, Japan, Study No. GHF-P-1385, March 3, 1993. MRID 45014705. Unpublished.

SPONSORS: Dow Chemical Japan Ltd., DowElanco Division, Seavans North, 2-1, Shibaura 1-chome, Minato-ku, Tokyo 105, Japan; Nichimen Corporation, 11-1, Nihonbashi 3-chome, Chuo-ku, Tokyo 103, Japan

EXECUTIVE SUMMARY: In a subchronic oral toxicity study (MRID 45014705), cyhalofop butyl was administered to 12 Fischer rats/sex/dose in the feed at doses of 0, 30, 300, 1000, or 3000 ppm for males and females. The mean estimated compound intake for males was 0, 1.719, 17.43, 60.5, or 189.5 mg/ kg/day, respectively, and for females was 0, 1.958, 19.64, 65.3, or 199.6 mg/kg/day, respectively.

All animals survived to study termination; no clinical signs were observed. There were no effects on body weight, food consumption, or food efficiency. There were no treatment-related effects on ophthalmologic or neurotoxicity parameters. There were no toxicologically significant effects on hematologic endpoints.

Liver changes suggestive of adaptive hypertrophy included increased absolute liver weights in the three highest male dose groups (22-67%) and in the 2 highest female dose groups (14 and 24%, respectively). Relative liver weights were comparably increased (13-72%). Such changes also included hepatocellular swelling with eosinophilic granules in these male groups and the 3000 ppm females. A modest increase in alkaline phosphatase activity in the highest male dose group is suggestive of cholestasis which would be compatible with a finding of adaptive hypertrophy resulting from xenobiotic chemical administration. However, the severity of the

histopathologic changes in the highest male dose group together with the ~70% increase in liver weight (absolute and relative) may indicate changes beyond simply an adaptive effect.

Absolute kidney weights were somewhat increased in the 3 highest male dose groups (6.6-13%) and slightly in the 2 highest female groups (3.9 and 5.3%). Relative kidney weights were also increased in these groups. Correlated gross changes in the kidneys included darkening in all of the highest dose males and females but at 1000 ppm, only 1/12 each in the male and female groups showed this effect. Mild deposition of lipofuscin pigment was observed in the renal proximal tubular cells of all rats in the highest dose male and female groups. Male rats at the highest dose also had severely decreased acidophilic bodies of these same cells; at 1000 ppm, 8/12 male rats had mild hyaline droplet degeneration of these cells. The deposition of lipofuscin pigment in 3000 ppm rats of both sexes is considered a toxicologically significant and adverse effect; the mild hyaline droplet degeneration at 1000 ppm in male rats may be a precursor to lipofuscin deposition but its significance is unclear.

Under the conditions of this study, the subchronic toxicity LOAEL is 3000 ppm for male (189.5 mg/kg/day) and female (199.6 mg/kg/day) rats, based on kidney toxicity in both sexes (lipofuscin pigment deposition in the proximal tubule cells), and possible liver toxicity in males. The NOAEL is 1000 ppm for male (60.5 mg/kg/day) rats and female (65.3 mg/kg/day) rats.

This subchronic oral toxicity study in rats is classified as **Acceptable/Guideline** and satisfies the [OPPTS: 870.3100(§82-1)] Subdivision F guideline requirements.

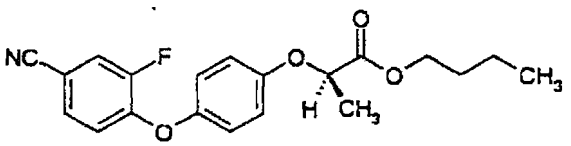
COMPLIANCE: Signed and dated GLP, Quality Assurance, Flagging, and Data Confidentiality statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test compound: Cyhalofop butyl

   Description: Off-white powder
   Lot No.: AGR 284267
   Purity: 97.4%
   Stability of compound: Stable in a dark and cold environment
   CAS No: 122008-85-9
   Structure:



2. Vehicle and/or positive control: Acetone

3. Test animals

Species: Rat
Strain: Specific-pathogen-free Fischer (F344/DuCrj)
Age/weight at study initiation: 5 weeks; males: 94 - 105 g; females: 78 - 86 g
Source: Charles River Japan, Inc., Kanagawa
Housing: 3 rats/cage in cages with stainless steel mesh bottoms, 31 x 44 x 23 cm
Diet: Certified diet MF Mash (Oriental Yeast Co., Tokyo), given *ad libitum*
Water: Well water, put through precipitating and sedimentation procedures and sterilized with hypochlorous acid and exposed to UV light, provided *ad libitum*
Environmental conditions:
Temperature: 24 ± 2 °C.
Humidity: 55 ± 15%
Air changes: 15 X per hour
Photoperiod: 12 hours on, 12 hours off
Acclimation period: One week

B. STUDY DESIGN

1. In life dates

Start: Males: 4/16/91; Females: 4/23/91
End: Males: 7/18/91; Females: 7/24/91

2. Animal assignment

TABLE 1. Study design

Concentration in diet (ppm)	Dose (mg/kg/day)		Number of rats	
	Male	Female	Male	Female
0	0	0	12	12
30	1.719	1.958	12	12
300	17.43	19.64	12	12
1,000	60.5	65.3	12	12
3,000	189.5	199.6	12	12

Data taken from p. 11 and Text Table 1 , p. 24, MRID 45014705.

3. Dose selection rationale

Dose selection and group size were based on the results of a prior 2-week range finding feeding study (IET 90-0161) at levels of 0, 100, 300, 1000, 3000, and 10,000 ppm using 6 male and 6 female rats per dose group. At 10,000 ppm, the males displayed a slight depression of food consumption and food efficiency resulting in lower body weight gain. Absolute and relative liver weights were increased in both sexes at 1000

ppm and above. Relative liver weights were increased in males at 300 ppm. Enlarged livers were observed in both sexes at 3000 and 10,000 ppm and only in males at 1000 ppm. No significant changes were observed at 100 ppm in either sex.

4. Diet preparation and analysis

The test diet preparation was performed once prior to start of treatment and twice during the treatment period. The test substance was dissolved in acetone and then incorporated into a premixture. The premixture was blended with the remaining basal diet by a mixer SS-501 (Kanto Kongoki Industrial Co., Ltd., Ohara-cho, Itabashi-ku, Tokyo) to the needed concentrations. The control diet was also mixed with acetone and prepared in the same manner; the acetone was evaporated from all diet concentrations in a draft chamber after which they were sealed in plastic bags and stored at about 4 °C in the dark until use. The diets given the animals were changed twice a week.

Samples for each dose level were taken from the top, middle, and bottom of the mixer for determination of homogeneity and concentration prior to treatment initiation. Concentration was determined in the samples from the middle of the mixer. No mention was made of stability sampling. The samples were also monitored for possible contamination.

Results –
Homogeneity analysis: Coefficients of variation were within 4.1% for each dose diet sampled from the top, middle, and bottom of the mixer.

Stability analysis: No mention was made of testing nor were results provided.

Concentration analysis: The average concentration of the test substance in 3 batches of test diets were 29±1.5, 287±5.5, 961±7.8, and 2829±14.1 ppm. The nominal values were 30, 300, 1000, and 3000 ppm, respectively. Every value measured was within a range of 94 to 97% of the nominal concentration.

5. Statistical analysis

Multiple comparison tests (Dunnett's or Scheffe's method) were used for body weight, food consumption, urine volume and specific gravity, hematology, blood biochemistry, and organ weights. Mann-Whitney's U test was used for urinalysis results other than volume and specific gravity, while Fisher's exact probability test was used for clinical signs, mortality, ophthalmology, and pathology results. Significance of the differences between control and treated groups were estimated at the 5% and 1% levels.

C. METHODS

1. Observations

Animals were observed at least once daily for clinical signs and daily for mortality. Detailed clinical observation was performed at least weekly.

2. Body weight

Body weight was recorded weekly during the study and before autopsy at terminal sacrifice.

3. Food consumption and compound intake

Food consumption was determined weekly by measurement of total amount consumed for a period of 3 consecutive days. Daily food consumption per animal in each cage was calculated by dividing the three-day total by 3 days and by the number of animals per cage. Group mean food consumption was calculated from daily food consumption per animal in each cage. Group mean test material intake was calculated from food consumption, nominal dose level, and body weight. Group mean food efficiency was calculated from the ratio of mean body weight gain to mean food consumption and expressed as a percentage.

4. Ophthalmoscopic examination

Ophthalmoscopic examinations were conducted on all animals of both sexes prior to treatment and on all survivors of both sexes in the control and highest dose groups at 13 weeks of treatment.

5. Blood was collected under ether anesthesia from the posterior vena cava after overnight starvation of all surviving rats after 13 weeks' treatment. The CHECKED (X) parameters were examined.

a. Hematology

X		X	
X	Hematocrit (HCT)*	X	Leukocyte differential count*
X	Hemoglobin (HGB)*	X	Mean corpuscular HGB (MCH)
X	Leukocyte count (WBC)*	X	Mean corpusc. HGB conc.(MCHC)
X	Erythrocyte count (RBC)*	X	Mean corpusc. volume (MCV)
X	Platelet count*	X	Reticulocyte count
	Blood clotting measurements*[a]		Blood cell morphology
	(Activated thromboplastin time)		
	(Clotting time)		
	(Prothrombin time)		

*Required for subchronic studies based on Subdivision F Guidelines.

b. Clinical chemistry

X	ELECTROLYTES	X	OTHER
X	Calcium*	X	Albumin*
X	Chloride*	X	Albumin/globulin ratio
	Magnesium	X	Blood creatinine*
X	Phosphorus*	X	Blood urea nitrogen*
X	Potassium*	X	Total cholesterol
X	Sodium*	X	Globulins
		X	Glucose*
	ENZYMES	X	Total bilirubin
X	Alkaline phosphatase (ALK)	X	Total serum protein*
	Cholinesterase (ChE)		Triglycerides
X	Creatine phosphokinase		Serum protein electrophoresis
	Lactic acid dehydrogenase (LDH)		
X	Alanine aminotransferase (also SGPT)*		
X	Aspartate aminotransferase (also SGOT)*		
X	Gamma glutamyl transferase (GGT)		
	Glutamate dehydrogenase		

*Required for subchronic studies based on Subdivision F Guidelines.

6. Urinalysis

Urinalysis was conducted at 13 weeks' treatment on all animals.

X		X	
X	Appearance	X	Glucose
X	Volume	X	Ketones
X	Specific gravity		Bilirubin
X	pH	X	Blood
X	Sediment (microscopic)		Nitrites
X	Protein	X	Urobilinogen

7. Sacrifice and pathology

After 92 days of treatment for males and 91 days for females, the animals were starved overnight, given ether anesthesia, blood was drawn, and the animals were weighed, sacrificed, and necropsied. The CHECKED (X) tissues in the table below were collected and examined microscopically. The (XX) organs, in addition, were weighed. Schmorl method and Prussian blue staining were applied in order to identify brown pigments observed in renal proximal tubular cells. Additional samples of the liver and kidney were taken from certain male and female control and high-dose animals for future examination by electron microscopy if requested.

X	DIGESTIVE SYSTEM	X	CARDIOVASC./HEMAT.	X	NEUROLOGIC
	Tongue	X	Aorta*	X X	Brain*+
X	Salivary glands*	X X	Heart*	X	Periph. nerve*
X	Esophagus*	X	Bone marrow*	X	Spinal cord (3 levels)*
X	Stomach*	X	Lymph nodes*	X X	Pituitary
X	Duodenum*	X X	Spleen*	X	Eyes (optic n.)
X	Jejunum*	X X	Thymus*		
X	Ileum*				GLANDULAR
X	Cecum*		UROGENITAL	XX	Adrenal gland*
X	Colon*	X X	Kidneys*+	X	Lacrimal gland
X	Rectum*	X	Urinary bladder*	X	Mammary gland*
X X	Liver*+	X X	Testes*+	X	Parathyroids*
X	Pancreas*	X	Epididymides*	X X	Thyroids*
		X	Prostate*	X	Coagulation glands
	RESPIRATORY	X	Seminal vesicle*		
X	Trachea*	X X	Ovaries*		OTHER
X	Lung*	X	Uterus*	X	Bone*
	Nose (nasal turbinates)		Vagina	X	Skeletal muscle*
	Pharynx			X	Skin*
	Larynx			X	All gross lesions and masses*

*Required for subchronic studies based on Subdivision F Guidelines.
+ Organ weight required in subchronic and chronic studies.

II. RESULTS

A. OBSERVATIONS

No deaths occurred in any group of either sex. There were no significant differences between controls and treated groups in the incidence of clinical signs at any time during the treatment period.

B. BODY WEIGHT AND BODY WEIGHT GAIN

A statistically significant decrease in body weight was observed at week 4 in males of the high-dose group (3000 ppm), but after that, no differences from control values were reported for either sex.

C. FOOD CONSUMPTION AND COMPOUND INTAKE

1. Food consumption

Food consumption was equivalent to that of the control animals of both sexes throughout the treatment period.

2. Test compound intake

Test compound intake was estimated weekly based on nominal diet concentration, food consumption, and body weight, and the 13 week averages are given in Table 1.

3. Food efficiency

Food efficiency in treated animals of all groups of both sexes was equivalent to that of the control animals throughout the treatment period.

D. OPHTHALMOSCOPIC EXAMINATION

No statistically significant differences were observed in the incidence of findings between the 3000 ppm group of either sex and the corresponding control group.

E. BLOOD WORK

1. Hematology

Statistically significant decreases below control values were observed in the 3000 ppm male group for RBC and Hb, while there were statistically significant increases in MCV, MCH, and reticulocyte count as shown in Table 2. Similar trends were observed in these endpoints in the 1000 ppm male group, although the changes in values reached statistical significance only for the decrease in RBC and MCH.

No differences from control values were reported for other hematologic parameters in males, nor were there any statistically significant differences in any female treatment group for any hematologic endpoint.

TABLE 2. Clinical hematology parameters for male rats fed Cyhalofop Butyl for 13 weeks[a]

Parameter	Treatment group (ppm)				
	0	30	300	1000	3000
RBC ($\times 10^6$/cmm)	8.48	8.56	8.33*	8.15*(3.9)[a]	7.99**(5.8)
Hb (g/dL)	15.3	15.4	15.2	15.0	14.8** (3.3)
MCV (fL)	48.2	47.9	48.5	49.1	49.4* (2.5)
MCH (pg)	18.0	18.0	18.3	18.4* (2.2)	18.5** (2.8)
Retic (/1000 RBC±S.D.)	5±2	5±3	5±2	8±4	9±4* (80)

Data taken from Table 17-1, p. 72, MRID 45014705.
MCV = Mean corpuscular volume.
MCH = Mean corpuscular hemoglobin.
Retic = Reticulocyte.
S.D. = Standard deviation.
*Significantly different from control at 5% level.
**Significantly different from control at 1% level.
[a]Numbers in parentheses are the percent difference relative to untreated controls, calculated by the reviewer.

2. Clinical chemistry

Selected clinical biochemistry parameters are listed in Table 3. The alkaline phosphatase (ALK) activity in 3000 ppm male rats was significantly elevated 53%.

The total protein level was elevated by 3.7% in the high-dose male group. Albumin was significantly elevated in all male treatment groups in a dose-dependent manner and in the 1000 and 3000 ppm female groups. Globulin decreased significantly in the 300, 1000, and 3000 ppm male groups and in the high-dose female group. The albumin/globulin ratio increased in a statistically significant and dose-related manner in the 300, 1000 and 3000 ppm male groups and in the female 1000 and 3000 ppm groups.

While small but statistically significant changes were reported for aspartate aminotransferase (SGOT), alanine aminotransferase (SGPT), and phosphorus in the two highest male dose groups, SGOT in the 1000 ppm female group, and for blood urea nitrogen and cholesterol in the three highest male dose groups, these changes were insufficient to be biologically meaningful. No changes were seen in any other biochemistry endpoints in male or female rats.

TABLE 3. Clinical biochemistry parameters for rats fed Cyhalofop Butyl for 13 weeks

Parameter	Male treatment group (ppm)				
	0	30	300	1000	3000
ALK (U/L) S.D.	300±21	290±21	292±23	338** (13)[a] ±29	458** (53) ±27
SGOT (U/L) S.D.	63 ±7	65±9	63±5	56* (-11) ±5	52** (-18) ±5
SGPT (U/L) S.D.	20±2	20±3	21±2	17* (-15) ±4	16** (-20) ±1
BUN (mg/dL) S.D.	16.6±1.3	16.7±0.8	18.7** (13)±1.2	20.2** (22) ±1.5	21.9** (32)±1.2
Total protein (g/dL);S.D.	6.28±0.15	6.44±0.16	6.37±0.13	6.38±0.22	6.51** (3.7)±0.20
Albumin (g/dL) S.D.	3.08±0.08	3.20**(3.9)±0.07	3.31** (7.5)± 0.10	3.50** (14)± 0.12	3.74** (21)±0.11
Globulin (g/dL) S.D.	3.21±0.10	3.24±0.11	3.05** (-5.0)± 0.10	2.88** (-10)±0.15	2.77** (-14)± 0.13
Albumin/globulin ratio S.D.	0.96±0.03	0.99±0.03	1.09**(14)0.05	1.22** (27) 0.06	1.36** (42)± 0.06
Phosphorus (mg/dL) S.D.	4.6±0.5	4.4±0.3	4.9±0.7	5.1*±0.6 (11)	4.9*±0.3 (6.5)
Cholesterol (mg/dL) S.D.	40±3	42±6	34* (-15)±3	30** (-25)±3	30** (-25)±2
Parameter	**Female treatment group (ppm)**				
	0	**30**	**300**	**1000**	**3000**
SGOT (U/L) S.D.	54±5	55±3	56±3	60** (11)±6	58±5
Albumin (g/dL) S.D.	3.09±0.09	3.13±0.11	3.17±0.14	3.28** (6.2)±0.17	3.34** (8.1)±0.14
Globulin (g/dL) S.D.	3.09±0.20	3.05±0.14	3.16±0.19	2.99±0.18	2.87* (-7.1)±0.22
Albumin/globulin ratio S.D.	1.00±0.07	1.03±0.06	1.04±0.09	1.10* (10)±0.10	1.17** (17) ±0.09

Data taken from Tables 19-1 and -2 and 20-1, pp. 76-8, MRID 45014705.
S.D. = Standard deviation.
*Significantly different from control at 5% level.
**Significantly different from control at 1% level.
[a]Numbers in parentheses are the percent difference relative to untreated controls, calculated by the reviewer.

F. URINALYSIS

Slight but statistically significant dose-dependent increases in specific gravity were seen in the two highest male dose groups (1% and 1.4% respectively) as presented in Table 4. No differences from control values were seen in this or any other endpoint in the other male groups or in any female group.

TABLE 4. Specific gravity of urine in male rats fed Cyhalofop Butyl for13 weeks

	Males				
	0 ppm	30 ppm	300 ppm	1000 ppm	3000 ppm
Specific gravity	1.075±0.0009	1.078±0.006	1.081±0.007	1.086±0.010** (1)	1.090±0.007** (1.4)

Data taken from Table 15, p. 70, MRID 45014705.
**Significantly different from controls at 1% level.

G. SACRIFICE AND PATHOLOGY

1. Organ weight

The liver weights of 300, 1000, and 3000 ppm male rats were significantly increased relative to the control value in a dose-dependent manner (see Table 5). The liver weights relative to terminal body weights were also significantly increased as were group mean liver relative to brain weight ratios. The liver weights of the 1000 and 3000 ppm female dose groups were also significantly elevated but to a much lesser extent than the males as were their weights relative to body weight and to brain weight.

The kidney weights of the three highest male dose groups were also modestly but significantly increased over the control value. The increases in kidney weight relative to body weight ratio were significant only for the 1000 and 3000 ppm groups, but the percent increases in kidney to brain weight ratios were closely comparable to the increases in absolute kidney weight for the three dose groups. The kidney weights of the 1000 and 3000 ppm female groups were significantly increased. The kidney to body weight ratio of the 3000 ppm group was correspondingly increased; the 1000 ppm group mean was 3.2% higher but did not reach statistical significance. Kidney to brain weight ratios for these two female groups were elevated by 1.7 and 3.3%, respectively.

The spleen weights of the 1000 and 3000 ppm male groups were significantly decreased relative to controls. Corresponding decreases in spleen relative to body weight were seen and in spleen to brain weight ratios. Changes in absolute or relative spleen weights were not observed in the female groups, nor were such changes seen in any other organs in male or female rats.

TABLE 5: Liver, kidney and spleen weights, absolute and relative (to total body weight and to brain weight) of male and female rats fed cyhalofop butyl for 13 weeks[a]

Organ and terminal body weight	Male, dose (ppm)				
	0	30	300	1000	3000
Body weight (g)± S.D.	323±14	327±6	328±16	324±13	313±12
Liver: absolute (g)	7.34±0.31	7.75±.32	8.92** (22)[a]±0.69	10.54** (44)±0.68	12.23** (67)±0.88
Liver: Relative to body weight (ratio x100) ± S.D.	2.28±0.05	2.37±0.08	2.72** (19)±0.13	3.25** (43)±0.15	3.91** (72)±0.18
Liver: Relative to brain weight (ratio)[b]	3.775	3.970 (5.2)	4.593 (22)	5.394 (43)	6.330 (68)
Kidney: absolute (mg)± S.D.	1,870±60	1,883±78	1,993* (6.6)±112	2,078** (11)±95	2,120** (13)±206
Kidney: Relative to body weight (ratio)± S.D.	0.58±0.02	0.58±0.02	0.61±0.02	0.64** (10)±0.03	0.68** (17)±0.06
Kidney: Relative to brain weight (ratio)	0.9614	0.9647	1.026 (6.7)	1.063 (11)	1.097 (14)
Spleen: absolute (mg)± S.D.	580±25	558±27	560±29	544* (-6.2)±33	508** (-12)±38
Spleen: Relative to body weight (ratio)± S.D.	0.18±0.01	0.17* ±0.01	0.17±0.01	0.17** (-5.6)±0.01	0.16** (-11)±0.01
	Female, dose (ppm)				
	0	30	300	1000	3000
Body weight (g)	194±10	197±8	194±10	195±9	191±7
Liver: absolute (g)	4.42±0.26	4.47±0.22	4.54±0.24	5.05** (14)[a]±0.31	5.46** (24) ±0.31
Liver: Relative to body weight ratio × 100	2.29±0.12	2.27±0.09	2.35±0.08	2.59** (13)±0.13	2.85** (24)±0.10
Liver: Relative to brain weight (ratio)[b]	2.453	2.483	2.486	2.743 (12)	2.972 (21)

Data taken from Tables 23-1 to 23-4 and 24-1 to 24-4, pp. 82-89, MRID 45014705.
*Significantly different from control at the 5% level.
**Significantly different from control at the 1% level.
[A]Numbers in parentheses are the percent difference relative to untreated controls, calculated by the reviewer.
[B]Organ to brain weight ratios calculated by the reviewer.

2. Gross pathology

Darkened livers were observed in 12/12 males and females exposed to 3000 ppm of the test substance and in 7/12 males and 2/12 females of the 1000 ppm groups (Table 6). Livers were enlarged in 12/12 of the 3000 ppm males, 9/12 in the highest dose females and 6/12 males (all significantly increased over controls), and 1/12 females of the 1000 ppm groups. Kidneys were darkened in all 3000 ppm males and females and at 1000 ppm, in 1/12 each in the male and female groups. Other gross changes were observed but were not significantly increased over the incidence in corresponding control groups.

TABLE 6: Incidences of selected compound-related gross changes in male and female rats fed cyhalofop butyl for 13 weeks[a]

Endpoint/concentration (ppm)	Male, n = 12				
	0	30	300	1000	3000
Darkened liver	0	0	0	7**	12**
Enlarged liver	0	0	0	6**	12**
Accentuated lobular pattern	0	0	0	0	1
Darkened kidneys	0	0	0	1	12**
	Female, n = 12				
Darkened liver	0	0	0	2	12**
Enlarged liver	0	0	0	1	9**
Darkened kidneys	0	0	0	1	12**

Taken from Tables 21 and 22, pp. 80-81, MRID 45014705.
**Significantly different from control at the 1% level.

3. Microscopic pathology

Compound-related microscopic changes occurring in the livers and kidneys of male and female rats are shown in Table 7. Statistically significant incidences of hepatocellular swelling with minute eosinophilic granules were reported in 300 ppm (4/12, mild), 1000 (12/12, mild to severe) and 3000 ppm males (12/12, severe). Similar, but all mild, changes were seen in 1000 ppm (1/12) and 3000 ppm (12/12) females. The hepatocytes around the central vein were generally more severely affected than those at the periportal region of the lobule, although in the 3000 ppm males, the change was evident throughout the lobules. The eosinophilic granules in affected hepatocytes extended throughout the cytoplasm.

Mild deposition of brown pigment in proximal tubular cells of the kidneys was seen in 12/12 each of the 3000 ppm male and female groups. The pigment was identified as lipofuscin by a positive reaction in the Schmorl method and negative results in Prussian blue staining. These changes were accompanied in male rats by severely decreased acidophilic bodies of the proximal tubular cells at 3000 ppm (12/12) and by mild hyaline droplet degeneration of proximal tubular cells only in the 1000 ppm group (8/12). Other microscopic changes were seen in male and female rats but were not different in incidence from the corresponding control groups.

TABLE 7: Incidences of selected compound-related microscopic changes in male and female rats

Endpoint/concentration (ppm)	Male, n = 12				
	0	30	300	1,000	3000
Liver: hepatocellular swelling with minute eosinophilic granules					
Mild	0		4	3	0
Moderate		0	0	7	0
Severe			0	2	12
Total			4*	12*	12**
Kidney: Increased brown pigment deposition of proximal tubule cells					
Mild					12
Moderate	0	0	0	0	0
Severe					0
Total					12**
Decreased acidophilic bodies of proximal tubular cells					
Mild					0
Moderate	0	0	0	0	0
Severe					12
Total					12**
Hyaline droplet degeneration of proximal tubular cells					
Mild				8	
Moderate	0	0	0	0	0
Severe				0	
Total				8**	
Endpoint/concentration (ppm)	**Female, n = 12**				
	0.00	**30**	**300**	**1,000**	**3000**
Liver: Hepatocellular swelling with minute eosinophilic granules					
Mild					12
Moderate	0	0	0	0	0
Severe					0
Total					12**
Kidney: Increased brown pigment deposition of proximal tubule cells					
Mild	0	0	0	0	12
Moderate					0
Severe					0
Total					12**

Data taken from Tables 25 and 26, pp. 90-91, and Appendices 20 and 21, pp. 175-191, MRID 45014705.
*Significantly different from control at the 5% level.
**Significantly different from control at the 1% level.

III. DISCUSSION

A. DISCUSSION

In a subchronic toxicity study, cyhalofop butyl was administered to 12 Fischer rats/sex/dose in the feed at doses of 0, 30, 300, 1000, or 3000 ppm for males and females. The mean estimated compound intake in males was 0, 1.719, 17.43, 60.5, or 189.5 mg/kg/day, respectively, and in females was 0, 1.958, 19.64, 65.3, or 199.6 mg/kg/day, respectively.

All animals survived to study termination without evidence of toxic signs. No changes in body weight or food consumption or efficiency were noted. No ophthalmologic abnormalities were observed in the control or highest dose groups at termination.

Male rats in the 3000 and 1000 ppm dose groups showed suggestive evidence of a slight anemia, although the changes, while statistically significant, were less than 6% different from control values and of no toxicological significance. The study authors considered the changes to be treatment-related and indicative of anemia, but the reviewer does not consider them to be of toxicologic significance.

Blood biochemistry tests revealed changes indicative of mild dehydration in that albumin levels were significantly increased in all male and the two highest female dose groups with resulting increases in calculated globulin levels and albumin/globulin ratios. These changes correlated with slightly increased urine specific gravity in the 1000 and 3000 ppm male groups. These changes are not considered biologically or toxicologically meaningful by the reviewer despite the study authors' conclusion that there was a treatment-related disordering of plasma protein levels. A statistically significant increase in SGOT activity in the 1000 ppm female group was isolated and is therefore considered incidental to treatment. The study authors interpreted increases in BUN to be evidence of disordered renal function, but the reviewer finds them to be within the range of biological variation and not toxicologically significant. Other small changes in blood biochemistry endpoints are not considered biologically or toxicologically significant.

Another change in blood biochemistry included a mild, statistically significant increase in alkaline phosphatase activity in the highest male dose group (53%) is consistent with cholestasis resulting from hypertrophy. (A small increase of 13% in the 1000 ppm males was too small to be biologically meaningful.) The increase at 3000 ppm was correlated with dose-related increases in absolute and relative liver weights in males in the 300 ppm and above dose groups and hepatocellular swelling with eosinophilic granules in these dose groups. Females in the 1000 and 3000 ppm dose groups had increases in absolute and relative liver weights and the 3000 ppm group likewise showed hepatocellular swelling with eosinophilic granules. The study authors interpreted the changes in liver weights, alkaline phosphatase activity in males, and histopathological and gross changes to be evidence of liver toxicity, but the reviewer views the significance of the liver changes in this study as likely primarily or solely an adaptive hypertrophy associated with increased metabolic activity engendered by test substance ingestion. However, the severity of these changes in all the highest-dose males coupled with absolute and relative

liver weight increases of about 70% over control values and elevated alkaline phosphatase activity may indicate changes beyond a mere adaptive response to a xenobiotic chemical in the highest dose male group.

Renal changes included darkening in all highest-dose male and female animals, somewhat increased absolute and relative kidney weights in the two highest male and female dose groups, and histopathologic changes of concern in the 3000 ppm male and female groups. Mild deposition of lipofuscin pigment was observed in the proximal tubule cells of all highest dose animals of both sexes; this is considered a toxicologically significant and adverse effect. Mild hyalin droplet degeneration was observed in 8/12 of the 1000 ppm males but in no female animals. The study authors interpreted this change as evidence for increased lysosomes in proximal tubule epithelium and a precursor to lipofuscin deposition which results from accumulation of subcellular membrane degradation products in lysosomes. The reviewer assesses this change to be of unclear significance. The 3000 ppm male group also showed severe depletion of acidophilic bodies in the proximal tubule cells in all animals.

Dose-dependent decreases in absolute and relative spleen weights in the two highest male dose groups appear to be treatment-related, but the significance is unclear as there were no histopathological or gross correlates. Statistically significant decreased relative spleen weight in the 30 ppm male dose group and increased absolute brain weight in the 1000 ppm female group appear to be incidental as there was no dose-dependency in either case.

Under the conditions of this study, the subchronic toxicity LOAEL is 3000 ppm (189.5 mg/kg/day) for male rats based on kidney (lipofuscin pigment deposition in the proximal tubule cells) and possibly liver toxicity and 3000 ppm (199.6 mg/kg/day) for female rats, based on kidney toxicity. The NOAEL for male and female rats is 1000 ppm (males: 60.5 mg/kg/day; females: 65.3 mg/kg/day).

B. STUDY DEFICIENCIES

One major deficiency was noted in this study; no assessment of the stability of the test substance in the diet was made. A minor deficiency is that a required measurement of blood clotting time were not performed. The interpretation of results does not appear to be affected by these deficiencies.

DATA EVALUATION RECORD

CYHALOFOP-BUTYL
(XRD-537 nBu)

STUDY TYPE: SUBCHRONIC ORAL TOXICITY STUDY - MOUSE
[OPPTS 870.3100 (§82-1a)]
MRID 45000412

Prepared for

Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
1921 Jefferson Davis Highway
Arlington, VA 22202

Prepared by

Chemical Hazard Evaluation Group
Toxicology and Risk Analysis Section
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Work Assignment No. 01-81U

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Disclaimer

This review may have been altered subsequent to the contractors' signatures above.

Oak Ridge National Laboratory, managed by UT-Battelle, LLC, for the U.S. Dept. of Energy under contract no. DE-AC05-00OR22725.

CYHALOFOP BUTYL **Subchronic Oral Toxicity Study [OPPTS 870.3100 (§82-1a)]**

EPA Reviewer: John Whalan, Date 5-9-01
EPA Work Assignment Manager: SanYvette Williams-Foy, D.V.M., Date 5/31/01
Registration Action Branch 2 (7509C)
TXR No: 0050348

DATA EVALUATION RECORD

STUDY TYPE: Subchronic Oral Toxicity Study in Mice [OPPTS 870.3100 (§82-1a)]

DP BARCODE: D268553 SUBMISSION CODE: S
P.C. CODE: 082583 TOX. CHEM. NO.: None given

TEST MATERIAL (PURITY): Cyhalofop butyl (purity 98.2 %)

SYNONYMS: Propanoic acid: 2-(4-(4-cyano-2-fluorophenoxy)-phenoxy)-n-butyl ester, (R(+)); XRD-537 nBu

CITATION: Corley, R.A., K.T. Haut, and L.G. Lomax (1991). XRD-537 NBU: Four-week and 13-week dietary toxicity studies in CD-1 mice. Toxicology Research Laboratory, Health and Environmental Sciences, Dow Chemical Company, Midland, MI 48674. Laboratory Project Study ID DR-0298-8876-002 and DR-0298-8876-002A. March 7, 1991. MRID 45000412. Unpublished.

SPONSOR: Dow Chemical Company, Midland, MI

EXECUTIVE SUMMARY: In a three-month subchronic oral toxicity study (MRID 45000412), XRD-537 nBu (lot number not stated, purity 98.2%) was fed to groups of 10 male and 10 female mice at targeted concentrations of 0, 1 (males only), 3, 10, 30, or 100 (females only) mg/kg body weight/day. In addition, groups of 5 male and 5 female mice were fed targeted concentrations of 0, 10 (males only), 30, 100, or 350 (females only) mg/kg/day for four weeks. There were no treatment-related clinical signs, deaths, or effects on body weight, food consumption, hematology, or clinical chemistry. Absolute and relative (to body weight) liver weights were increased at least 30% in both sexes at the higher doses after thirteen weeks, but these increases were associated with hepatocellular hypertrophy and are considered to be an adaptive, rather than toxicological, response. Absolute and relative kidney weights were increased 10% in females after thirteen weeks, and these increases were judged to be biologically significant even in the absence of microscopic correlates.

Under the conditions of this study, the subchronic oral toxicity LOAEL for XRD-537 nBu was not determined in either sex. The NOAEL is ≥30 mg/kg/day for males and ≥100 mg/kg/day for females.

This study is classified as **Acceptable/Guideline** and satisfies the guideline requirement for a subchronic oral study in mice [OPPTS 870.3100 (§82-1a)].

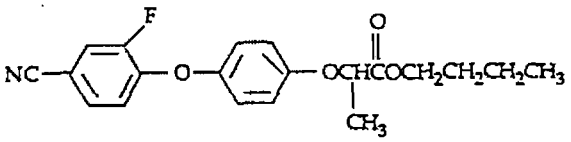
COMPLIANCE: Signed and dated Good Laboratory Practice, Quality Assurance, Data Confidentiality, and Flagging statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: XRD-537 nBu

Description: solid
Lot Number: not provided
Purity: 98.2 %
Stability of compound: At least 32 days in rodent feed
CAS #: 122008-85-9
Structure:



2. Vehicle and/or positive control

Purina Certified Chow #5002

3. Test animals

Species: Mice
Strain: CD-1
Age at study initiation and weight at study week 0: ~7 weeks; males: 26.9-33.6 g; females: 18.8-24.6 g
Source: Charles River Breeding Laboratory, Portage, MI
Housing: singly in suspended stainless steel cages with wire-mesh floors
Diet: Purina Certified Chow #5002, *ad libitum*
Water: tap water, *ad libitum*.
Environmental conditions:
Temperature: Not provided, but designated as "adequate" and regulated for the species
Humidity: Not provided, but designated as "adequate" and regulated for the species
Air changes: Not provided
Photoperiod: Not provided, but designated as "adequate" and regulated for the species
Acclimation period: 14 days

B. STUDY DESIGN

1. In-life dates

Start: not stated; end: not stated

2. Animal assignment

Animals were assigned by a computer-generated randomization scheme based on body weight to the groups shown in Table 1.

TABLE 1. Study design

Targeted dose (mg/kg/day)	13-week sacrifice		4-week sacrifice	
	Number of animals			
	Males	Females	Males	Females
0	10	10	5	5
1	10	–	–	–
3	10	10	–	–
10	10	10	5	–
30	10	10	5	5
100	–	10	5	5
350	–	–	–	5

Data taken from text on p. 11, MRID 45000412

3. Dose selection rationale

Dose levels were selected to determine organ weight and microscopic changes in the target tissues of liver and kidneys in order to make comparisons with previous four-week and subchronic studies conducted with the methyl ester of XRD-537 and to aid in selecting dose levels for subsequent chronic toxicity studies.

4. Test material preparation and analysis

Test diets were prepared weekly by adding a minimal amount of acetone to the test material and mixing it into the feed. The high-dose diet was serially diluted with the appropriate amount of feed to create the lower-dose diets. The initial concentration of the test material in the diets was calculated from historical body weight and food consumption data; thereafter, the most recent body weight and food consumption data were used. Reference samples (one/dose/sex/mix) were stored at ambient temperatures. Analysis of the diets to verify test material concentration was conducted during weeks 1, 4, 9, and 12.

Results -

Homogeneity analysis: The mixing method was validated, with samples ranging from 93 to 103% of the target concentration.

Stability analysis: The test material was found to be stable for at least 32 days in rodent chow, with recoveries ranging from 96 to 102%.

Concentration analysis: During the study dietary concentrations of the test material ranged from 89% to 124% of the target dose level, with the exception of the 3 and 10 mg/kg/day concentrations for females during week 9, which were 330% and 1100%

of the target dose. The source of error was not clear. Absence of XRD-537 nBu in the control diet was confirmed.

5. Statistics

Four week data: Means and standard deviations were determined for food consumption. Body weight and organ weight values were evaluated by Bartlett's test ($p \leq 0.01$, except for liver weight, which used $p \leq 0.05$). In-life body weights were evaluated among treated groups using a repeated measures analysis of variance for time and dose ($p \leq 0.10$). If the time-dose interaction was significant, the analysis was repeated for each dose against the controls and a Bonferroni correction for multiple comparisons ($p \leq 0.05$) was used. Terminal body and organ weights were analyzed using a parametric analysis of variance followed by a Dunnett's test ($p \leq 0.05$).

Thirteen-week data: Means and standard deviations were determined for food consumption and leukocyte differential counts. Body weight, organ weight, clinical chemistry, and hematology data were evaluated by Bartlett's test for equality of variances ($p \leq 0.01$). Based on the outcome, parametric or nonparametric analysis of variance ($p \leq 0.10$) was performed, followed respectively by Dunnett's test ($p \leq 0.05$) or the Wilcoxon Rank-Sum test ($p \leq 0.05$) with a Bonferroni correction ($p \leq 0.02$).

C. METHODS

1. Observations

All animals were observed at least once daily during the work week for treatment-related signs. An additional observation was made each day of the work week and twice on weekends and holidays for morbidity and mortality. Detailed clinical examinations were conducted prior to the study start and weekly thereafter.

2. Body weight

Body weights were recorded weekly.

3. Food consumption and compound intake

Food consumption was recorded weekly.

4. Food efficiency

Food efficiency was not reported.

5. Ophthalmoscopic examination

At necropsy, eyes were examined by visual inspection of the cornea, lens and other internal components by placement of a moistened glass slide on the corneal surface using fluorescent light.

6. Blood was collected at thirteen weeks via orbital sinus puncture from anesthetized mice prior to necropsy. It was not stated whether the animals were fasted prior to blood collection. The CHECKED (X) parameters were examined for all animals.

a. Hematology

X	Hematocrit (HCT)*	X	Leukocyte differential count*
X	Hemoglobin (HGB)*		Mean corpuscular HGB (MCH)
X	Leukocyte count (WBC)*		Mean corpusc. HGB conc.(MCHC)
X	Erythrocyte count (RBC)*		Mean corpusc. volume (MCV)
X	Platelet count*		Reticulocyte count
	Blood clotting measurements*		
	(Thromboplastin time)		
	(Clotting time)		
	(Prothrombin time)		

* Recommended for subchronic studies, per Subdivision F Guidelines.

b. Clinical chemistry

	ELECTROLYTES		OTHER
X	Calcium*	X	Globulin
X	Chloride*		Albumin/globulin ratio (A/G)
	Magnesium	X	Albumin*
X	Phosphorus*	X	Blood urea nitrogen (BUN)*
X	Potassium*	X	Creatinine*
X	Sodium*	X	Glucose*
			Phospholipids
	ENZYMES	X	Total bilirubin*
X	Alkaline phosphatase (ALP)	X	Total protein (TP)*
	Cholinesterase (ChE)	X	Total cholesterol
	Creatine phosphokinase	X	Triglycerides
	Lactic acid dehydrogenase (LDH)		
X	Serum alanine aminotransferase (ALT also SGPT)*		
X	Serum aspartate aminotransferase (AST also SGOT)*		
	Gamma glutamyl transferase (GGT)		

* Recommended for subchronic studies, per Subdivision F Guidelines

7. Urinalysis

Urinalysis was not performed.

8. Sacrifice and pathology

Mice were sacrificed under methoxyflurane anesthesia. At the thirteen-week sacrifice, the CHECKED (X) tissues were collected and examined histologically for all mice in the control and high-dose groups, as well as the animal sacrificed in a moribund condition during the study. In addition, the (XX) organs were weighed. For the other dose groups, microscopic examination of tissues was done only for liver, kidneys, lungs, and all gross lesions. At the four-week sacrifice, microscopic examination of tissues was done for liver, kidneys, and gross lesions only, and the liver and kidneys were weighed.

	DIGESTIVE SYSTEM		CARDIOVASC./HEMAT.		NEUROLOGIC
X	Tongue	X	Aorta*	XX	Brain*
X	Salivary glands*	XX	Heart*	X	Peripheral nerve
X	Esophagus*	X	Bone marrow*	X	Spinal cord (3 levels)
X	Stomach*	X	Lymph nodes*	X	Pituitary*
X	Duodenum*	X	Spleen*	X	Eyes (optic nerve)
X	Jejunum*	X	Thymus*		
X	Ileum*				GLANDULAR
X	Cecum*		UROGENITAL	X	Adrenal glands*
X	Colon*	XX	Kidneys*	X	Lacrimal gland
X	Rectum*	X	Urinary bladder*	X	Mammary gland
XX	Liver*+	XX	Testes*+	X	Parathyroid*
X	Gall bladder	X	Epididymides	X	Thyroid*
X	Pancreas*	X	Prostate	X	Coagulating gland
		X	Seminal vesicle		
	RESPIRATORY	X	Ovaries		OTHER
X	Trachea*	X	Uterus*	X	Bone
X	Lung*	X	Vagina	X	Skeletal muscle
X	Nose	X	Cervix	X	Skin
	Pharynx			X	All gross lesions and masses*
X	Larynx				

* = Required for subchronic studies based on Subdivision F Guidelines.
+ = Organ weight required in subchronic studies based on Subdivision F Guidelines.

II. RESULTS

A. OBSERVATIONS

1. Toxicity

No treatment-related clinical observations were reported.

2. Mortality

One male in the 10 mg/kg/day group was sacrificed moribund on day 79. The cause of death was not evident after gross and microscopic examination.

B. BODY WEIGHT AND WEIGHT GAIN

At the four-week sacrifice, males in the 100 mg/kg/day group had a slight but statistically significant increase (6%, $p \leq 0.02$) in body weight compared to controls. No treatment-related effects on body weight were noted in females. At thirteen weeks, no treatment-related effects on body weight were seen in either sex. On test day 80, weight decreases of 15-19% from the previous week were seen in all groups of males. A problem was found with the water delivery system and was corrected. All animals appeared to recover prior to termination of the study.

TABLE 2. Initial and final mean body weights and body weight gains of mice fed XRD-537 nBu for 4 or 13 weeks

Parameter	Target dose (mg/kg/day)						
	0	1	3	10	30	100	350
Males							
4-week sacrifice							
Initial (day -4) body weight (g)	30.2	–	–	29.9	30.7	30.2	–
Final body weight (g)	36.6	–	–	35.4	35.1	38.7	–
Body weight gain (g)[a]	6.4	–	–	5.5	4.4	8.5	–
13-week sacrifice							
Initial (day -4) body weight (g)	30.0	30.1	29.7	30.4	29.5	–	–
Final body weight (g)	39.9	38.9	37.6	39.1	39.5	–	–
Body weight gain (g)[a]	9.9	8.8	7.9	8.7	10.0	–	–
Females							
4-week sacrifice							
Initial (day -4) body weight (g)	22.0	–	–	–	22.3	22.3	22.0
Final body weight (g)	26.1	–	–	–	26.7	27.0	28.8
Body weight gain (g)[a]	4.1	–	–	–	4.4	4.7	6.8
13-week sacrifice							
Initial (day -4) body weight (g)	22.5	–	22.6	22.1	22.1	22.3	–
Final body weight (g)	29.3	–	28.1	29.3	28.6	29.6	–
Body weight gain (g)[a]	6.8	–	5.5	7.2	6.5	7.3	–

[a]Calculated by the reviewer
Data taken from Tables 9-10, 19-20, pp. 40-41, 51-53, MRID 45000412

C. FOOD CONSUMPTION AND COMPOUND INTAKE

1. Food consumption

At four weeks, the 100 mg/kg/day males had slightly increased food consumption (6.3 g/day compared to 5.9 g/day for controls; no statistical analysis was performed). No effects were seen in the females. Decreases in food consumption in all the male groups occurred from days 75-80 as a result of the problem with the water delivery system, but the animals appeared to recover prior to termination of the study. No effects on food consumption were seen in females.

2. Compound consumption

The targeted doses for the treated groups are given in Table 1. The calculated doses in the main study (submitted by registrant in May, 2001) were 0, 1.01, 3.02, 10.2 or 30.6 in males, and 0, 2.96, 10.40, 30.8, or 104 mg/kg/day in females.

3. Food efficiency

Food efficiency was not calculated by the study authors.

D. OPHTHALMOSCOPIC EXAMINATION

Examination at necropsy revealed no treatment-related effects.

E. BLOOD WORK

1. Hematology

There were no treatment-related effects on hematology parameters.

2. Clinical chemistry

Clinical chemistry parameters were determined only for the mice sacrificed at thirteen weeks (Table 3). The mean alkaline phosphatase activity of the 30 mg/kg/day males was significantly increased (83%, $p \leq 0.05$) compared to controls, and the mean serum cholesterol was increased (43%, $p \leq 0.05$) in the 100 mg/kg/day females. The only other significant difference from controls was an increase in alanine aminotransferase (23%, $p \leq 0.05$) for the 3 mg/kg/day females.

TABLE 3. Selected clinical chemistry parameters for mice fed XRD-537 nBu for 13 weeks

Parameter	Target dose (mg/kg/day)					
	0	1	3	10	30	100
Males						
Alanine aminotransferase (mu/mL)	39	41	39	42	61	–
Alkaline phosphatase (mu/mL)	92	104	98	118	168* (83)[a]	–
Aspartate aminotransferase (mu/mL)	55	56	53	53	64	–
Cholesterol (mg/dL)	94	80	79	86	82	–
Females						
Alanine aminotransferase (mu/mL)	26	–	32* (23)	28	26	30
Alkaline phosphatase (mu/mL)	141	–	139	141	137	135
Aspartate aminotransferase (mu/mL)	53	–	59	56	51	53
Cholesterol (mg/dL)	65	–	61	71	76	93* (43)

*Significantly different from controls ($p \leq 0.05$)
[a]Numbers in parentheses are percent different from controls, calculated by the reviewer
Data taken from Tables 27 and 29, pp. 60 and 62, MRID 45000412

F. URINALYSIS

Urinalysis was not performed.

G. SACRIFICE AND PATHOLOGY

1. Organ weight

At the four-week sacrifice, absolute liver weight was significantly increased by 56% and 88% in the 30 and 100 mg/kg/day males, respectively (Table 4). The relative (to body weight) liver weight was significantly increased by 26%, 54%, and 78% in the 10, 30, and 100 mg/kg/day males. In females, absolute liver weight was increased by 40% and 135% in the 100 and 350 mg/kg/day groups, respectively, with corresponding increases of 39% and 108% in the relative liver weight. The 350 mg/kg/day females also had a significant increase (26%, $p \leq 0.05$) in absolute kidney weight.

At the thirteen-week sacrifice, absolute liver weight was significantly increased by 12% and 30% in the 10 and 30 mg/kg/day males, respectively, accompanied by a 14% and 32% increase in the relative liver weight. Relative kidney weight was significantly increased (14%) in the 1 mg/kg/day males, but not in the higher dose groups. In females, absolute liver weight was significantly increased (38%) in the 100 mg/kg/day group, and relative liver weight was significantly increased by 10, 16, and 38%, respectively, in the 10, 30, and 100 mg/kg/day groups. Absolute and relative kidney weight were each significantly increased by 10% in the 100 mg/kg/day group.

TABLE 4. Selected mean organ and organ/body weights of mice fed XRD-537 nBu for 4 or 13 weeks

Parameter	Target dose (mg/kg/day)						
	0	1	3	10	30	100	350
Males							
4-week sacrifice							
Final body weight (g)	36.5	–	–	35.7	36.8	38.6	–
Liver weight (g)	2.145	–	–	2.629	3.340* (56)	4.026* (88)[a]	–
Liver/body weight (g/100)	5.860	–	–	7.368* (26)	9.030* (54)	10.439* (78)	–
13-week sacrifice							
Final body weight (g)	39.1	38.4	36.8	38.4	38.4	–	–
Liver weight (g)	2.169	2.106	2.101	2.437* (12)	2.826* (30)	–	–
Liver/body weight (g/100)	5.563	5.479	5.702	6.356* (14)	7.350* (32)	–	–
Kidney weight (g)	0.619	0.699* (13)	0.605	0.683	0.672	–	–
Kidney/body weight (g/100)	1.594	1.824* (14)	1.643	1.784	1.746	–	–
Females							
4-week sacrifice							
Final body weight (g)	26.1	–	–	–	26.5	26.3	29.6* (13)
Liver weight (g)	1.527	–	–	–	1.933	2.142* (40)	3.588* (135)
Liver/body weight (g/100)	5.859	–	–	–	7.272	8.153* (39)	12.172* (108)
Kidney weight (g)	0.384	–	–	–	0.408	0.404	0.484* (26)
Kidney/body weight (g/100)	1.470	–	–	–	1.537	1.540	1.633
13-week sacrifice							
Final body weight (g)	29.0	–	27.8	28.2	28.2	29.0	–
Liver weight (g)	1.528	–	1.509	1.623	1.717	2.106* (38)	–
Liver/body weight (g/100)	5.259	–	5.434	5.770* (10)	6.086* (16)	7.258* (38)	–
Kidney weight (g)	0.419	–	0.412	0.440	0.438	0.460* (10)	–
Kidney/body weight (g/100)	1.449	–	1.483	1.567	1.554	1.592* (10)	–

*Significantly different from control ($p \leq 0.05$)
[a]Numbers in parentheses are percent different from controls, calculated by the reviewer
Data taken from Tables 13, 14, 31, and 32, pp. 44, 45, 64, and 65, MRID 45000412

2. Gross pathology

At the four-week sacrifice, enlarged livers were seen in 3/5 30 mg/kg/day males, 5/5 100 mg/kg/day males, and 5/5 350 mg/kg/day females. Pale foci were seen in the liver of at least one male from each treated group and in 3/5 females of the 350 mg/kg/day group. At the thirteen-week sacrifice, pale foci were seen in the liver of 1/10 males in each of the 3 and 30 mg/kg/day groups. There were no other treatment-related observations. A female in the 3 mg/kg/day group had a focus on one lung lobe, but this was considered incidental.

3. Microscopic pathology

At the four-week sacrifice, microscopic examination revealed hepatocellular hypertrophy in all mice from all dose groups. The hypertrophy was multifocal (centrilobular distribution) in all males of the 10 mg/kg/day group and all females of the 30 mg/kg/day group, and diffuse (panlobular distribution) in all males in each of the 30 and 100 mg/kg/day groups and all females in each of the 100 and 350 mg/kg/day groups. The enlarged hepatocytes contained granular eosinophilic cytoplasm. Multifocal hepatocellular necrosis (individual cells or groups of adjacent cells) was accompanied by inflammation in 2/5 males and 5/5 males at 30 and 100 mg/kg/day, respectively, and 2/5 females and 4/5 females at 100 and 350 mg/kg/day, respectively. Additionally, 3/5 females in the 350 mg/kg/day group had increased multifocal mitotic figures.

At the thirteen-week sacrifice, multifocal hepatocellular hypertrophy was seen in 9/10 males at 10 mg/kg/day and 10/10 females at 30 mg/kg/day. Diffuse hepatocellular hypertrophy was seen in 1/10 males and 10/10 males at 10 mg/kg/day and 30 mg/kg/day, respectively, and in 10/10 females at 100 mg/kg/day. The hypertrophic hepatocytes contained granular eosinophilic cytoplasm. Additionally, 2/10 males at 30 mg/kg/day and 1/10 females at 100 mg/kg/day had foci of hepatocellular necrosis accompanied by inflammatory cells.

III. DISCUSSION

A. DISCUSSION

No treatment-related deaths occurred in the study, and no treatment-related clinical signs were reported. At the four-week sacrifice, body weight and food consumption were unaffected by treatment. The slight increase in body weight seen in high-dose males was likely due to increased food intake and liver enlargement. The dose-related increases in absolute and relative liver weights in both sexes at four weeks were associated with hepatocellular hypertrophy, which was found in all mice at all doses. Kidney weight was increased 26% in high-dose females, but there were no microscopic correlates and this finding is not considered toxicologically significant.

There were no treatment-related effects on body weight, food consumption, hematology, or clinical chemistry parameters after thirteen weeks. Although mean alkaline phospha-

tase activity was increased in the high-dose males, this is considered secondary to the hepatocellular hypertrophy. Serum cholesterol was increased 43% in 100 mg/kg/day females, but this is also considered to be a secondary effect. As in the four-week mice, the increased absolute and relative liver weights at the higher doses in both sexes were associated with hypertrophy. Focal necrosis, which was prevalent in the mice sacrificed at four weeks, was seen in only a few mice from the high dose groups after 13 weeks. Since hypertrophy is an adaptive, rather than toxicological response, the increased liver weights are not considered to be toxicologically significant. Low-dose males had increased absolute and relative kidney weights, but this effect was not seen at the higher doses. The high-dose females had a 10% increase in kidney weight. In the absence of any microscopic correlates, this is not considered toxicologically significant.

Under the conditions of this study, the subchronic oral toxicity LOAEL for XRD-537 nBu was not determined in either sex. The NOAEL is ≥30 mg/kg/day for males and ≥100 mg/kg/day for females.

This study is classified as **Acceptable/Guideline** and satisfies the guideline requirement for a subchronic oral study in mice [OPPTS 870.3100 (§82-1a)].

B. STUDY DEFICIENCIES

The OPPTS recommended blood clotting measurement was not performed. The air change frequency in the animal room was not reported. These deficiencies would not be expected to affect the conclusions of the study.

DATA EVALUATION RECORD

CYHALOFOP BUTYL
(XRD-537 BE)

STUDY TYPE: 90-DAY ORAL TOXICITY - MOUSE [OPPTS 870.3100 (§82-1)]
MRID 45014706

Prepared for

Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
1921 Jefferson Davis Highway
Arlington, VA 22202

Prepared by

Chemical Hazard Evaluation Group
Toxicology and Risk Analysis Section
Life Sciences Division
Oak Ridge National Laboratory
Oak Ridge, TN 37831
Task Order No. 01-81T

Primary Reviewer: Sylvia S. Talmage, Ph.D., D.A.B.T.	Signature: Date:	Sylvia S. Talmage FEB 0 7 2001
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Disclaimer

This review may have been altered subsequent to the contractor's signatures above.

Oak Ridge National Laboratory, managed by UT-Battelle, LLC, for the U.S. Department of Energy under contract number DE-AC05-00OR22725.

CYHALOFOP BUTYL, technical **90-Day Oral Toxicity Study [OPPTS 870.3100 (§82-1)]**

EPA Reviewer: John Whalan, Date 5-7-01
Registration Action Branch 2 (7509C)
EPA Work Assignment Manager: S. Williams-Foy, D.V.M., Date 5/31/01
Registration Action Branch 2 (7509C)
TXR No: 0050348

DATA EVALUATION RECORD

STUDY TYPE: Subchronic Oral (Dietary) Toxicity Study - Mouse [OPPTS 870.3100 (§82-1)]

DP BARCODE: D268553
P.C. CODE: 082583

SUBMISSION CODE: None
TOX. CHEM. NO.: None

TEST MATERIAL (PURITY): XRD-537 BE (Cyhalofop butyl; 97.4% purity)

SYNONYMS: XDE-537; XRD-537; XRD-537 n-butyl ester; (R)-(+)-n-butyl-2-(4-(2-fluoro-4-cyanophenoxy) phenoxy) propanoate

CITATION: Harada, T., Ebino, K., Odanaka, Y., Maita, K. (1993) XRD-537 BE: 13-Week oral subchronic toxicity study in mice. The Institute of Environmental Toxicology, Suzuki-cho 2-772, Kodaira-shi, Tokyo 187, Japan. Study No. GHF-P-1390, March 3, 1993. MRID 45014706. Unpublished.

SPONSOR: Dow Chemical Japan, Ltd., DowElanco Division, Sevans North, 2-1 Shibaura 1-chome, Minato-ku, Tokyo 105, Japan; Nichimen Corporation, 11-1, Ninobashi 3-chome, Chuo-ku, Tokyo 103, Japan

EXECUTIVE SUMMARY: In a 90-day dietary study (MRID 45014706), XRD-537 BE (Lot # AGR 284267, 97.4% purity) was administered to 12 ICR (Crj:CD-1) mice/sex/dose at dietary levels of 0, 3, 30, 100, or 300 ppm (males: 0.0, 0.4, 3.6, 12.4, or 37.5 mg/kg/day; females: 0.0, 0.4, 4.3, 14.1, or 41.4 mg/kg/day). Body weights, food consumption, and clinical observations were recorded. Urinalysis and ophthalmoscopic examinations were conducted. At study termination, the mice were sacrificed and blood was collected for hematology and clinical chemistry studies. Organ weights were recorded and gross and microscopic examinations were conducted.

All mice survived to terminal sacrifice. There were no treatment-related clinical signs of toxicity and no effects on body weight, food consumption, food efficiency, ophthalmology, urinary parameters, or hematology or clinical chemistry parameters in any group.

Increases in liver weight accompanied by histological correlates of hepatocellular swelling with minute eosinophilic granules and focal necrosis were considered treatment related but not toxicologically significant. In male mice in the 100 and 300 ppm groups, increased absolute liver weights (26% and 39%, respectively; $p<0.01$) and liver weights relative to body weight (23% and 37%, respectively; $p<0.01$) were accompanied by hepatocellular swelling with eosinophilic granules in all 12 mice in both treatment groups ($p<0.01$) and slight to moderate focal necrosis in

3/12 male mice (not statistically significant) and 5/12 male mice ($p<0.05$), in the respective groups. These effects were not observed in the male control group. The absolute liver weight of male mice in the 30 ppm group was increased by 12% and was unaccompanied by histological correlates. The relative liver weight of females in the 100 ppm group was increased by 10% ($p<0.05$) and absolute and relative liver weights of females in the 300 ppm group were increased by 26% and 21%, respectively. Although the incidence and severity of focal necrosis were elevated in female mice in the 300 ppm group [6/12 (graded slight and moderate) compared with 2/12 (slight) in the control group], neither incidences nor severity were clearly dose related in female mice.

Absolute and relative kidney weights were not affected in male mice. Compared with the female control group, there were increases in absolute and relative kidney weights in female mice in the 30 ppm group [14% ($p<0.01$) and 12% ($p<0.05$), respectively], 100 ppm group (both 20%; both $p<0.01$), and 300 ppm group (23% and 18%, respectively; both $p<0.01$). Increased kidney weights were accompanied by swelling of proximal tubular cells in females in the 100 and 300 ppm groups [incidences of 4/12 ($p<0.05$) and 6/12 ($p<0.01$), respectively]. The increased kidney weights in female mice in the 100 and 300 ppm groups accompanied by swelling of kidney tubules was considered an adverse effect.

The LOAEL is 100 ppm in female mice (14.1 mg/kg/day) based on enlarged kidneys accompanied by swelling of the proximal tubule cells. A LOAEL for male mice was not attained. The NOAEL for female mice is 30 ppm (4.3 mg/kg/day); the NOAEL for male mice is ≥300 ppm (37.5 mg/kg/day).

This study is considered to be **Acceptable/Guideline** as a 90-day feeding study and fulfills FIFRA guideline requirements for a subchronic oral toxicity study [870.3100 (§82-1)] in the mouse.

COMPLIANCE: Signed and dated GLP, Quality Assurance, Data Confidentiality, and Flagging statements were provided.

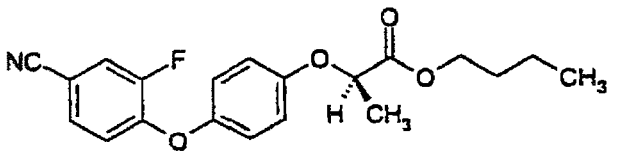
I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: XRD-537 BE (Cyhalofop butyl)

   Description: off-white powder
   Lot No.: AGR 284267
   Purity: 97.4%
   Stability of compound: Stable for 123 days in rodent feed.
   CAS No.: 122008-85-9

Structure:



2. Vehicle and/or positive control

The test material was dissolved in acetone prior to mixing with the feed. The acetone was allowed to evaporate before feeding commenced. The control diet was treated in the same manner.

3. Test animals

Species: Mouse
Strain: ICR (Crj:CD-1)
Age and weight at study initiation: 5 weeks; males: 25.0-29.4 g; females: 22.4-26.9 g
Source: Charles River Japan, Inc., Shimofurusawa, Atsugi-shi, Kanagawa
Housing: Three per sex per cage in aluminum cages with wire-mesh floors. Cages were rotated among four tiers every four weeks.
Diet: Certified MF Mash (Oriental Yeast Co., Ltd., Tokyo) was available *ad libitum*.
Water: filtered and sterilized well water (hypochlorous acid and UV radiation) was available *ad libitum*.
Environmental conditions:
Temperature: 22.9-24.0°C
Humidity: 50-59%
Air changes: 15/hour
Photoperiod: 12 hour light/12 hour dark
Acclimation period: 10 days

B. STUDY DESIGN

1. In life dates

Start: May 20, 1991; end: August 27, 1991

2. Animal assignment

Following selection of animals for body weight and absence of clinical signs, animals were assigned to the test groups in Table 1 by means of a computer-generated random method based upon body weight.

TABLE 1: Study design					
Test group	Dose Level (ppm)	Mean dose to animal (mg/kg/day)		Number of Animals	
		Male	Female	Male	Female
1 (Control)	0	0	0	12	12
2	3	0.3674	0.4433	12	12
3	30	3.576	4.251	12	12
4	100	12.44	14.11	12	12
5	300	37.50	41.39	12	12

Data taken from p. 9 and Text Table 1, p. 22, MRID 45014706.

3. Dose selection rationale

Doses were based on a previous 2-week range-finding study (IET 90-0163) in which 6 male and 6 female mice were administered XRD-537 BE in the feed at dietary levels of 0, 30, 100, 300, or 1000 ppm. Effects included enlarged livers with significantly increased absolute and relative weights in both sexes in the 1000 ppm group. Absolute and relative liver weights were significantly increased in males and females in the 300 ppm group and in males in the 100 ppm group. There were no treatment-related effects in either sex in the 30 ppm group. Based on these results, the highest dietary concentration was set at 300 ppm with lower concentrations of 100, 30, and 3 ppm.

4. Test material preparation and analysis

Test diets were prepared twice (prior to and during treatment) by weighing appropriate amounts of the test material for each level, dissolving the material in acetone, and then mixing with the basal diet to make premixes. Each premix was blended with the remaining amount of the basal diet with an automatic mixer (SS-501, Kanto Kongoki Industrial Co. Tokyo, Japan). The control diet was similarly treated with acetone. The acetone in the diets was allowed to dry prior to storage. The test diets were sealed in plastic bags and stored in aluminum containers in the dark at 4°C until needed. Feed containers were replenished twice a week.

During preparation of the first batch of diets, two samples were taken from each of three locations (upper, middle, and bottom) of the mixer of all dose levels and analyzed for homogeneity on the day of sampling. All six samples from each dose level were analyzed for concentration. Samples from the second batch of diet were analyzed for concentration (2 replicates per dose level). Analyses of stability in feed were not conducted as part of this study, but were reported in an earlier study (MRID 45000412). In that study, samples were stored under conditions similar to the present study and subjected to chemical analysis after 6, 13, 32, and 123 days.

Results -

Homogeneity analysis: Concentrations in the upper, middle, and bottom of the mixer from the 3 ppm nominal dietary mixture ranged from 2.9-3.2 ppm; the coefficient of variation was 1.8%. Ranges of concentrations in the upper, middle, and bottom of the mixer from the 30, 100, and 300 ppm nominal concentrations were 28-29, 92-95, and 279-284 ppm, respectively. The coefficients of variation were 0.0, 1.6, and 0.6%, respectively. Two samples from the control diet contained <0.3 ppm.

Concentration analysis: The six samples from each dose level analyzed for homogeneity also served as samples for concentration analyses. Mean concentrations in the 3, 30, 100, and 300 ppm nominal concentrations were 3.1±0.1, 29±0.5, 93±1.5, and 282±1.8 ppm, respectively. Mean concentrations in the second batch for the respective dose levels were 3.0, 30, 94, and 284 ppm (standard deviations were not supplied). Overall mean concentrations ranged from 94-103% of target.

Stability analysis: Samples of the test chemical in the diet were stable for up to 32 days (recoveries of 96-102%); recovery on day 123 was 91%.

The analytical data indicated that the mixing procedure was adequate (CVs were ≤1.8%), that the variance between nominal and actual dosage to the animals (94-103%) was acceptable, and that the test substance was stable in the feed for time periods relevant to the present study.

5. Statistics

For multiple comparisons of parameters such as body weight, food consumption, urine specific gravity, hematology, clinical chemistry, and organ weights, individual dose groups were compared to the control group with Dunnett's or Scheffe's method. The Mann-Whitney *U* test was applied to urine parameters except for specific gravity. Fisher's exact probability test was used for incidences of clinical signs, mortality, ophthalmology signs and pathology. Levels of significance of 5% and 1% were flagged.

C. METHODS

1. Observations

All animals were observed daily for clinical signs of toxicity and mortality. Detailed clinical observations were performed weekly.

2. Body weight

All animals were weighed weekly beginning with the first day of treatment. Animals were also weighed at terminal sacrifice.

3. Food and water consumption, food efficiency, and compound intake

Food consumption for each cage was measured once a week over a consecutive three-day period. Daily food consumption per animal was calculated by dividing the total weekly food consumption by the number of animals in each cage. These values were averaged to attain group means. Food efficiency was calculated weekly. Group mean food efficiency for each group was calculated as: (g mean body weight gain)/(g mean food consumption) x 100. The average daily compound intake was calculated weekly based on food consumption, nominal dose level, and body weight. The 13 weekly values were averaged.

4. Ophthalmoscopic examination

Ophthalmoscopic examinations were conducted pretreatment on all animals and at week 13 on control animals and animals in the 300 ppm dietary group. Examinations were performed with a halogen opthalmoscope.

5. Blood was collected at the termination of treatment. Blood was collected from the posterior vena cava under ether anesthesia. Information on fasting prior to blood collection was not provided. The CHECKED (X) parameters were examined.

a. Hematology

X		X	
X	Hematocrit (HCT)*	X	Leukocyte differential count*
X	Hemoglobin (HGB)*	X	Mean corpuscular HGB (MCH)*
X	Leukocyte count (WBC)*	X	Mean corpuscular HGB concentration (MCHC)*
X	Erythrocyte count (RBC)*	X	Mean corpuscular volume (MCV)*
X	Platelet count*		Reticulocyte count
	Blood clotting measurements*		Heinz body determination
	(Thromboplastin time)		RBC morphology
	(Fibrinogen)		Methemoglobin
	(Prothrombin time)		

* Recommended for subchronic studies based on OPPTS 870.3100 Guidelines.

b. Clinical chemistry

X	**ELECTROLYTES**	X	**OTHER**
X	Calcium		Albumin*
	Chloride		Albumin/globulin ratio
	Magnesium		Blood creatinine*
	Phosphorus	X	Blood urea nitrogen*
	Potassium*	X	Total cholesterol*
	Sodium*		Globulins
		X	Glucose*
	ENZYMES		Total bilirubin
X	Alkaline phosphatase (ALK)*	X	Total serum protein (TP)*
	Cholinesterase (ChE)		Triglycerides
	Creatine phosphokinase		Serum protein electrophoresis
	Lactic acid dehydrogenase (LDH)		Phospholipids
X	Serum alanine amino-transferase (also SGPT)*		
X	Serum aspartate amino-transferase (also SGOT)*		
	Gamma glutamyl transpeptidase (GGT)*		
	Glutamate dehydrogenase		

* Recommended for subchronic studies based on OPPTS 870.3100 Guidelines.

6. Urinalysis*

During week 13, urinalysis was conducted on fresh samples collected from all animals by pressing on the abdomen. The CHECKED (X) parameters were examined.

X		X	
	Appearance	X	Glucose
	Volume	X	Ketones
X	Specific gravity		Bilirubin
X	pH	X	Blood
	Sediment (microscopic)		Nitrites
X	Protein	X	Urobilinogen

*Not required for subchronic studies by OPPTS 870.3100 Guidelines

7. Sacrifice and pathology

A complete necropsy was conducted on all animals that died prematurely or were sacrificed moribund. All animals were sacrificed on schedule by exsanguination under ether anesthesia. Gross morphological examination was conducted on major tissues and organs of all mice. The CHECKED (X) tissues and organs were collected and examined microscopically. The head with tongue, nasal cavity, pharynx, and larynx and the vagina were collected but not examined microscopically. The DOUBLE-CHECKED (XX) tissues/organs were also weighed. Samples of the livers from two animals in the male and female control and 300 ppm groups were fixed and embedded in plastic for possible electron microscope examination. The liver from one male in the 30 ppm was additionally treated with oil red O and reacted with PAS in order to identify the contents of vacuoles in the hepatocytes.

X	DIGESTIVE SYSTEM	X	CARDIOVASC./HEMAT	X	NEUROLOGIC
	Tongue	X	Aorta*	XX	Brain (several sections)*+
X	Salivary glands*	XX	Heart*+	X	Peripheral. nerve*
X	Esophagus*	X	Bone marrow*	X	Spinal cord (3 levels)*
X	Stomach*	X	Lymph nodes*	XX	Pituitary*
X	Duodenum*	XX	Spleen*+	X	Eyes (optic nerve)*
X	Jejunum*	XX	Thymus*+		
X	Ileum*				GLANDULAR
X	Cecum*		UROGENITAL	XX	Adrenal glands*+
X	Colon *	XX	Kidneys*+		Lacrimal gland
X	Rectum*	X	Urinary bladder*	X	Mammary gland*
XX	Liver*+	XX	Testes*+	XX	Parathyroids*
X	Gall bladder*	X	Epididymides*+	XX	Thyroids*
X	Pancreas*	X	Prostate*		Zymbal gland
		X	Seminal vesicle*		Harderian gland
	RESPIRATORY	XX	Ovaries*+		
X	Trachea*	X	Uterus*+		OTHER
X	Lung*		Vagina	X	Bone (femur with joint)
	Nose*			X	Skeletal muscle
	Pharynx*			X	Skin*
	Larynx*			X	All gross lesions and masses*

* Required for subchronic studies based on OPPTS 870.3100 Guidelines.
+ Organ weight required in subchronic and chronic studies.

II. RESULTS

A. OBSERVATIONS

1. Toxicity

Males in the 300 ppm group showed a significant increase ($p<0.05$) in hair loss. Incidences in the 0, 3, 30, 100, and 300 ppm groups were 0, 1, 0, 0, and 4, respectively. Respective incidences for females were 1, 3, 3, 5, 4 (not significant). There were no other statistically significant differences between the control and treated groups.

2. Mortality

All animals survived the treatment period.

B. BODY WEIGHT AND WEIGHT GAIN

Group mean body weights for selected weeks and total body weight gains are shown in Table 2. There was no effect of treatment on body weights or body weight gains for males or females in any treatment group. For males, mean final body weights in the treatment groups ranged from 100 to 107% of the mean control value, and for females, mean final body weights of the treatment groups ranged from 99 to 105% of the mean control value.

TABLE 2. Selected week group mean body weights (g), total body weight gains (g), mean daily food consumption (g), and overall food efficiency (%) of mice fed XRD-537 BE for 13 weeks[a]

Week of study or parameter	Exposure concentration (ppm)				
	0	3	30	100	300
Males					
0	27.4±1.2	27.3±1.2	27.4±1.1	27.4±1.1	27.4±1.2
7	41.7±3.2	40.9±3.1	43.1±3.0	41.5±2.3	42.1±3.4
13	45.6±4.5	45.6±4.2 (100)	48.7±4.7 (107)	46.6±2.8 (102)	47.1±5.6 (103)
Total weight gain	18.2	18.3 (100)	21.3 (117)	19.2 (106)	19.7 (108)
Mean food consumption	4.9	4.8	4.9	5.0	5.1
Food efficiency	3.9	4.1	4.7	4.2	4.1
Females					
0	24.5±1.2	24.5±1.2	24.5±1.2	24.5±1.2	24.6±1.2
7	32.4±2.5	31.7±3.2	32.5±2.1	31.5±2.3	32.6±3.1
13	35.9±3.1	36.9±4.9 (103)	36.4±3.6 (101)	35.6±2.8 (99)	37.8±5.4 (105)
Total weight gain	11.4	12.4 (109)	11.9 (104)	11.1 (97)	13.2 (116)
Mean food consumption	4.4	4.5	4.4	4.3	4.4
Food efficiency	2.8	2.9	2.9	2.8	3.2

Data taken from Tables 5-8, 11, and 12, pp. 53-56, 59, and 60, MRID 45014706.
[a] Values in parenthesis are percent of control values.

C. FOOD CONSUMPTION AND COMPOUND INTAKE

1. Food consumption and food efficiency

There was no effect of treatment on food consumption or food efficiency. Data are presented in Table 2.

2. Compound consumption

Compound consumption was calculated by the study authors and is presented in Table

D. OPHTHALMOSCOPIC EXAMINATION

No findings related to treatment were observed. One male in the control group had an opaque cornea at the 13-week examination. One female with "corectopia" of the left pupil was mistakenly allocated to the 3 ppm group. This abnormality did not affect the outcome of the study as no additional abnormalities related to treatment were observed.

E. BLOOD WORK

1. Hematology

There were no treatment-related changes in hematology parameters.

2. Clinical chemistry

There were no treatment-related changes in clinical chemistry parameters.

F. URINALYSIS

Compared with the control value, the urinary pH was lower in males in the 30, 100, and 300 ppm groups (Table 3). This parameter was not affected in female mice as pH values were uniformly distributed between pH 6.0 and 8.0 in all groups. Ketones were also significantly lower in males in the three highest dose groups compared with the control group ($p<0.05$; grade: negative), but treated males had the same grade (negative) as control and treated females.

TABLE 3. Urinalysis parameters of mice fed XRD-537 BE for 13 weeks

Parameter	Dietary concentration (ppm)				
	0	3	30	100	300
Males					
pH value					
6.0					1
6.5		1	1	4	5
7.0	1	3	4	4	2
7.5	5	3	6	2	3
8.0	6	5	1*	2**	1**
Ketone grade					
negative (-)	6	8	12*	12*	12*
slight (+)	4	2			
moderate (++)	2	2			
Females					
pH value					
6.0	1		3	1	
6.5	3	1	1	3	5
7.0	3	4	3	1	3
7.5	1	2	1	4	1
8.0	4	4	4	3	2
8.5		1			1
Ketone grade					
negative (-)	12	12	12	12	12

Data taken from Tables 15 and 16, pp. 63 and 64, MRID 45014706.
*Statistically significant, $p<0.05$.
**Statistically significant, $p<0.01$.

G. SACRIFICE AND PATHOLOGY

1. Organ weights

At the end of the treatment period, significant dose-related increases in absolute and relative (to body weight) liver weights were observed in male mice in the 100 and 300 ppm groups ($p<0.01$); the mean absolute liver weight in male mice in the 30 ppm group was also increased ($p<0.05$) (Table 4). Absolute liver weights in the 100 and 300 ppm groups of males were increased by 26 and 39% over the control value, respectively. The corresponding relative liver weights were increased by 23 and 37%. The relative liver weight of females in the 100 ppm group was increased by 10% over the control value ($p<0.05$) and the absolute and relative liver weights of females in the 300 ppm group were increased by 26 and 21% (both $p<0.01$).

Absolute and relative kidney weights in females in the 30, 100, and 300 ppm groups were significantly increased as compared with controls ($p<0.05$ or $p<0.01$). Increases in absolute kidney weight in the 3, 30, 100, and 300 ppm groups were 1, 14, 20, and 23%, respectively. Corresponding relative kidney weight increases were 12, 20, and 18%. Increases in kidney weights were not observed in male mice. No other changes in organ weights were dose related for either sex.

TABLE 4. Terminal body weights (g), absolute organ weights and relative organ weights (% of body weight) of mice fed XRD-537 BE for 13 weeks

Terminal body and organ weights	Dietary concentration (ppm)				
	0	3	30	100	300
Males					
Body weight	46.0±4.5	45.5±4.0	49.0±4.7	46.9±2.8	47.1±5.6
Liver					
absolute weight (g)	2.49±0.23	2.39±0.26	2.79±0.33*	3.14±0.18**	3.47±0.35**
relative weight	5.43±0.32	5.28±0.58	5.69±0.36	6.70±0.39**	7.41±0.64**
Kidney					
absolute weight (mg)	724±117	666±56	689±93	703±61	665±44
relative weight	1.58±0.28	1.47±0.10	1.42±0.22	1.50±0.11	1.43±0.19
Females					
Body weight	36.1±3.1	36.8±4.8	36.9±3.4	35.9±3.2	37.7±5.4
Liver					
absolute weight (g)	1.83±0.20	1.76±0.19	1.89±0.21	2.02±0.32	2.31±0.34**
relative weight	5.08±0.47	4.81±0.35	5.11±0.39	5.61±0.57*	6.13±0.38**
Kidney					
absolute weight (mg)	399±31	402±27	456±39**	478±44**	489±66**
relative weight	1.11±0.09	1.10±0.11	1.24±0.11*	1.33±0.09**	1.31±0.13**

Data taken from Tables 23 and 24, pages 73-80, MRID 45014706.
*Statistically significant, $p<0.05$.
**Statistically significant, $p<0.01$.

2. Gross pathology

Lesions observed grossly are summarized in Table 5. Treatment-related findings included dark-colored livers in males and females in the 300 ppm group (12/12 males and 12/12 females) and 100 ppm group (11/12 males and 10/12 females) and enlargement of the liver in the 300 ppm (11/12 males and 11/12 females) and 100 ppm groups (11/12 males and 3/12 females). There were no treatment-related lesions observed grossly in the 3 and 30 ppm groups.

TABLE 5. Incidences of macroscopic lesions observed in mice fed XRD-537 BE for 13 weeks

Organ/Lesion	Dietary concentration (ppm)				
	0	3	30	100	300
Males					
Liver					
dark color	0	0	0	11**	12**
enlargement	0	0	0	11**	11**
Females					
Liver					
dark color	0	0	0	10**	12**
enlargement	0	0	0	3	11**

Data taken from Tables 21 and 22, pages 71-72, MRID 45014706.
**Statistically significant, $p<0.01$.

3. Microscopic pathology

a. Non-neoplastic – Histopathologic findings are summarized in Table 6. In the liver, there was an increase in hepatocellular swelling with minute cytoplasmic eosinophilic granules in male and female mice in the 100 and 300 ppm groups. Incidences of this lesion were 12/12 in both sexes in the 300 ppm group and in males in the 100 ppm group (all $p<0.01$). The incidence in females in the 100 ppm group was 5/12 ($p<0.05$). This lesion was generally graded as moderate in males in the 100 ppm group and severe in males in the 300 ppm group; whereas, in females it was graded slight in the 100 ppm group and slight (6/12) and moderate (6/12) in the 300 ppm group.

Focal hepatocellular necrosis was observed in 3/12 males and 2/12 females in the 100 ppm group and 5/12 males and 6/12 females in the 300 ppm group. Only the incidence of 5/12 males in the 300 ppm group attained statistical significance ($p<0.05$). Incidences and severity (graded slight and moderate) were greater in animals in the 300 ppm group than in the 100 ppm group (100 ppm: 2 slight and 1 moderate; 300 ppm: 2 slight and 3 moderate). This lesion was also present in females in the control and lower dose groups: 2/12 in the control group (graded slight), 0/12 in the 3 ppm group, and 3/12 in the 30 ppm group (1 graded slight and 2 graded moderate), indicating no clear dose-response relationship for severity in females. Results of observa-

tions following staining of the liver of a male mouse in the 30 ppm group with oil red O and PAS were not provided.

Swelling of the proximal tubule cells of both kidneys was observed in 4/12 females in the 100 ppm group ($p<0.05$) and 6/12 females in the 300 ppm group ($p<0.01$). This lesion was generally graded slight (3/4 females in the 100 ppm group and 4/6 females in the 300 ppm group) with lesions in the remaining females graded moderate. This lesion was not observed in male mice. There were no other treatment-related lesions.

TABLE 6. Non-neoplastic microscopic changes in organs of mice fed XRD-537 BE for 13 weeks[a]

Organ: lesion	Dietary concentration (ppm)				
	0	3	30	100	300
Males					
Liver					
Hepatocellular swelling with minute eosinophilic granules	0	0	0	12**	12**
Focal necrosis	0	1	0	3	5*
Kidney					
Swelling, proximal tubular cells	0	0	0	0	0
Females					
Liver					
Hepatocellular swelling with minute eosinophilic granules	0	0	0	5*	12**
Focal necrosis	2	0	3	2	6
Kidney					
Swelling, proximal tubule cells	0	0	0	4*	6**

[a]Based on 12 mice/sex/group.
Data taken from Tables 25 and 26, pp. 81-85, MRID 45014706.
*Statistically significant, $p<0.05$.
**Statistically significant, $p<0.01$.

b) Neoplastic - There were no neoplastic findings.

III. DISCUSSION

A. DISCUSSION

There were no deaths during the study. There were no treatment-related signs of systemic toxicity or effects on body weight, food consumption, food efficiency, the eyes, or hematology or clinical chemistry parameters. Although a higher incidence of hair loss was noted in male mice in the high-dose group compared with the control group, this change was not considered treatment-related by the study authors. There were no histological lesions associated with hair loss. The reviewer agrees with the study authors and further notes that hair loss in females was fairly evenly distributed among treated groups.

There were no treatment-related changes in hematology or clinical chemistry parameters. The absence of ketones in the urine of male mice is not an effect as ketones are normally not present in the urine.

There was a dose-dependent increase in absolute liver weight in male mice in the 30, 100, and 300 ppm groups (12, 26, and 39%, respectively) and in relative liver weight in males in the 100 and 300 ppm groups. Liver weights of females in the 100 and 300 ppm dose groups were also affected, but liver weight was not affected in females in the 30 ppm group. The liver enlargement was observed grossly in the 100 and 300 ppm groups of both sexes as was the dark coloration of the livers. Histological examinations revealed hepatocellular swelling accompanied by formation/deposition of minute eosinophilic granules in male and female mice in the 100 and 300 ppm dose groups. Severity of the lesion in both sexes tended to be greater in the 300 ppm group than in the 100 ppm group. The incidence of focal hepatocellular necrosis was significantly increased only in males in the 300 ppm group, and both incidences and severity increased in a dose-dependent manner in both sexes in the 100 and 300 ppm groups. However, the highest severity rating was "moderate." When either incidences or severity of focal necrosis of the liver are considered across female dose groups, there is no clear dose-response relationship (incidences and severity were the same in the 0 and 100 ppm female groups). Liver hypertrophy with focal areas of necrosis is an adaptive response to chemical treatment and the reviewer does not consider this effect toxicologically significant.

Incidences of bilateral swelling of the proximal tubular cells of the kidney were treatment-related in female mice in the 100 and 300 ppm dose groups. This lesion was not present in the lower dose groups of female mice or in any group of male mice. Absolute and relative kidney weights were also significantly increased in females in these two groups. Although absolute and relative kidney weights were significantly increased in females in the 30 ppm group, proximal tubular swelling was not observed in this dose group. It should be noted that in a chronic/carcinogenicity study with the mouse administered up to 100 ppm in the diet for 78 weeks (MRID 45000418), kidney weights were not significantly increased for either sex at any observation time (beginning at 26 weeks), but tubular dilatation, chronic glomerulonephritis, and hyaline casts were significantly increased in female mice administered 100 ppm. Thus, the increased kidney weights observed in the present study may be transient, but the swelling of the tubule cells indicates the beginning of an adverse treatment-related effect on the kidneys.

The study authors considered the 30 ppm dietary concentration a "minimum toxic level" based on the dose-dependent increased absolute and relative kidney weight of female mice; the 3 ppm concentration was a "maximum no-effect level." Because there were no histological correlates in the kidney and no indication of a functional deficit, the reviewer did not consider the increased absolute kidney weight of the 30 ppm group toxicologically significant. Thus, the reviewer considered the 30 ppm concentration a NOAEL for effects on the kidney. Based on increased liver weights accompanied by eosinophilic granules, the study authors appeared to consider 100 ppm a LOAEL for effects on the liver. The liver hypertrophy (enlarged, dark-colored livers accompanied by hepatocellular swelling in both male and female mice and an increase in hepato-

cellular focal necrosis in male mice) is considered by the reviewer to be an adaptive response to chemical treatment. It should be noted that in a chronic/carcinogenicity study with the mouse (MRID 45014706), increased liver weight of up to 24% in males receiving 100 ppm XRD-537 BE in the diet was a transient effect. Therefore, the reviewer considers 300 ppm in the diet a NOAEL for effects on the liver.

The LOAEL is 100 ppm in female mice (14.1 mg/kg/day) based on increased absolute and relative kidney weights accompanied by swelling of the proximal tubule cells. A LOAEL for male mice was not attained. The NOAEL for female mice is 30 ppm (4.3 mg/kg/day); the NOAEL for male mice is ≥300 ppm (37.5 mg/kg/day).

B. STUDY DEFICIENCIES

Several required hematology and clinical chemistry parameters were not examined. These were: blood clotting measurements, potassium and sodium electrolytes, serum gamma glutamyl transpeptidase activity (GGT), serum albumin and blood creatinine. These deficiencies do not invalidate the study, particularly if these parameters were measured in a subchronic feeding study with the rat (MRID 45014705 or MRID 45000413).

Damage to the kidney may be associated with leakage of GGT from cells. However, GGT was not measured in this study. Measurement of additional clinical chemistry parameters would help to determine if functional effects on the kidney did occur.

The stability data for XRD–537-BE were not found in MRID 45000417 as stated by the study authors. However, sufficient stability data were located in MRID 45000412.

DATA EVALUATION RECORD

CYHALOFOP BUTYL
(XRD-537 BE)

STUDY TYPE: SUBCHRONIC ORAL TOXICITY - Dog (OPPTS 870.3150 [§82-1])
MRIDs 45000410, 45014707

Prepared for

Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
Jefferson Davis Highway
Arlington, VA 22202

Prepared by

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Task Order No.01-81 V, W

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Disclaimer

This review may have been altered subsequent to the contractor's signatures above.

Oak Ridge National Laboratory, managed by UT-Battelle, LLC.,for the U.S. Department of Energy under contract number DE-AC05-00OR22725

CYHALOFOP BUTYL **Subchronic Oral Study [OPPTS 870.3150 (82-1)]**

EPA Reviewer: John Whalan, Date 5-9-01
Registration Action Branch 2 (7509C)
EPA Secondary Reviewer: SanYvette Williams-Foy, D.V.M., Date 5/31/01
Registration Action Branch 2 (7509C)
TXR No: 0050348

DATA EVALUATION RECORD

STUDY TYPE: Subchronic Oral Toxicity- Dog [OPPTS 870.3150 (§82-1)]

DP BARCODE: D268553
P.C. CODE: 082583

SUBMISSION CODE: S
TOX. CHEM. NO.: n/a

TEST MATERIAL: cyhalofop butyl; purity ≥97%

SYNONYMS: R-(+)-n-Butyl-2-(4-(2-fluoro-4-cyanophenoxy)phenoxy)propionate; AGR 276541; propanoic acid, 2-(4-(4-cyano-2-fluorophenoxy)phenoxy)n-butyl ester (R(+));XRD-537nBu; XRD-537 BE

CITATION: Harada, T., et al. (1994). XRD-537 BE: 13-week oral subchronic toxicity study in dogs. Institute of Environmental Toxicology, Suzuki-cho 2-772, Kodaira-shi, Tokyo 187, Japan. Laboratory Study ID GHF-P-1389. February 1, 1994. MRID 45014707.

Mizell, M.J., K.T. Haut, and J.W. Crissman (1990) XRD-537nBu: Palatability and four-week dietary probe study in beagle dogs. Toxicology Research Laboratory, Health and Environmental Sciences, Dow Chemical Company, Midland, MI 48674. Laboratory Project Study ID DR-0298-8876-004. September 11, 1990. MRID 45000410. Unpublished.

SPONSOR: Dow AgroSciences LLC

EXECUTIVE SUMMARY: In a subchronic toxicity study (MRID 45014707) XRD-537 BE (Lot no. AGR295713, purity 97.1%) was administered in diet to four dogs/sex at doses of 0, 100, 500, or 2500 ppm. Average doses to animals were 0, 2.91, 14.7, or 75.2 mg/kg/day for males, and 0, 3.17, 15.6, or 79.4 mg/kg/day for females. No animals died during the study. The only treatment-related clinical sign observed was loose stool, occurring in 0/4, 0/4, 1/4, and 2/4 males in the control, 100, 500, and 2,500 ppm dose groups, respectively. Loose stools were observed in 0/4, 0/4, 4/4, and 2/4 females, in control, 100, 500, and 2500 ppm groups, respectively. All treated groups had body weights within 4% of controls throughout the study, but high dose males and females had cumulative body weight gains that were 85 and 88%, respectively of controls. Low and mid-dose groups had body weight gains comparable to controls throughout the study.

High dose females had significant decreases in erythrocytes and hemoglobin, 85-87% of control levels ($p<0.01$ and 0.05 respectively) along with non-significant decreases in hematocrit and increases in MCV, 88 and 104% of control levels, respectively. In high dose males, erythrocytes, hemoglobin, and hematocrit were nonsignificantly decreased to 84-86% of control levels. Platelets were increased in high dose females ($p<0.01$), and non-significantly increased in high dose males. Total bilirubin was significantly increased ($p<0.01$) in high dose males and females. Triglycerides were reduced in high dose males ($p<0.05$) and potassium was decreased in low and high dose females ($p<0.05$ and 0.01, respectively).

The gallbladder was distended in 3/4 mid-dose females and 4/4 and 3/4 high dose males and females, respectively, compared with 0/4 male and female controls, probably due to the use of low fat feed. The thymus was brown and/or atrophied in all high dose males and females, and one control male. Absolute and relative thymus weights were reduced to 44 and 50% of control levels, respectively, ($p<0.01$) in high dose females, and nonsignificantly reduced in high dose males. Relative liver weight was increased in high dose males to 120% of the control level ($p<0.01$), and nonsignificantly increased to 112% of the control level in high dose females. Increased cytoplasmic eosinophilia of hepatocytes was present in 4/4 high dose males and females compared with 0/4 male and female controls. Hyaline droplet degeneration of proximal tubular cells was found in 2/4 and 3/4 high dose males and females, respectively, and in no control animals. Follicular epithelial hypertrophy and pale colloid were found in thyroids of 2/4 high dose females, and in no controls.

Under the conditions of this study, the LOAEL for the systemic toxicity of XRD-537 BE in beagle dogs is 2500 ppm (75.2, and 79.4 mg/kg/day for males and females, respectively) based on brown and/or atrophied thymuses, and decreased thymus weight. The NOAEL is 500 ppm (14.7 and 15.6 mg/kg/day in males and females, respectively).

This subchronic toxicity study is classified as **Acceptable/Guideline.** This study does satisfy the Subdivision F requirements for a 90-day oral toxicity study in non-rodents [OPPTS 870.3150 (§82-1)].

COMPLIANCE: Signed and dated Quality Assurance, GLP, Data Confidentiality, and Flagging statements were provided.

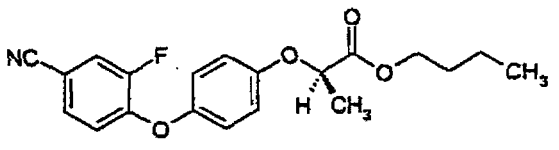
I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: XRD-537 BE

   Description: off-white powder
   Lot/Batch #: AGR295713
   Purity: 97.1%
   Stability of compound: said to be stable in a cold and dark environment
   CAS #: 122008-85-9

Structure:



Impurities: unknown

2. Vehicle and/or positive control

The test substance was administered in the diet. Control animals received food prepared in the same manner but lacking the test compound.

3. Test animals

Species/strain: Beagle
Age and weight at study initiation: approximately 5 months old, males 8.5-8.6 kg, females 8.1 kg
Source: Suwa Farm of CSK Experimental Animal Laboratory Co., Ltd., Toyoda, Suwa-shi, Nagano.
Housing: individually in stainless steel cages
Diet: certified diet DS, Oriental Yeast Co., Ltd., Azusawa, Itabashi-ku, Tokyo)
300 g/dog/day, moistened with 300 g water
Water: sterilized well water, *ad libitum*
Environmental conditions
Temperature: 24 ±2° C
Humidity: 55 ±10%
Air changes: 15 times per hour
Photoperiod: 12 hour light/dark cycle
Acclimation period: 30 days

B. STUDY DESIGN

1. In life dates

Start: April 8, 1992; end: July 16, 1992

2. Animal assignment

Dogs were assigned to the test groups in Table 1 by computerized randomization using stratified body weight.

TABLE 1: Study design					
Test group and dose level (ppm)		Number of animals per group		Average chemical intake (mg/kg/day)	
		Male	Female	Male	Female
Control	0	4	4	0	0
Low	100	4	4	2.91	3.17
Mid	500	4	4	14.7	15.6
High	2500	4	4	75.2	79.4

Data taken from text table, p. 10, MRID 45014707.

3. Rationale for dose selection

Dose selection was based on a palatibility/range finding study (MRID 45000410). See Appendix A.

4. Dose preparation and analysis

A specified amount of the test substance for each dose level was dissolved in acetone and then mixed with part of the feed in a plastic bag to make a pre-mixture. The pre-mixture was then blended with the remaining part of the feed by an SS-501 mixer to obtain the desired concentration and a homogeneous distribution of the test substance. The control diet was treated with acetone and prepared in the same manner. A 30 minute period was allowed for evaporation of acetone before the prepared diets were sealed in plastic bags, which were placed in a plastic container and stored in a dark 4°C room until used. Diets were later transferred to aluminum containers where they were stored at room temperature for up to 15 days immediately preceding use.

Stability: Stability of the test material in the feed was tested on samples from the 100 ppm diet prepared during the study. The samples were stored in the dark at 4°C for 59 days, followed by 15 days at room temperature in the animal room, and then for one day mixed with water (1:1, w/w) in a feeding jar. Stability of the enantiomer ratio (R/S) of the test material in the feed was also tested at the 500 ppm dose level on samples stored under the same conditions listed above.

Homogeneity: Homogeneity of the test substance in the diet was monitored on 50 g samples taken from the top, middle, and bottom of the mixer at each dose level prior to treatment.

Concentration: Concentration of the test material at each dose level was checked on 50 g samples taken from the middle of each mixer prior to initiation of treatment.

Results –

Stability: Analysis of samples after storage revealed that the test substance was present at 92-100% of the initial concentration. Enantiomer ratio (R/S) was 99.1/1.0, and remained unchanged during the storage period.

Homogeneity: Homogeneity of the test material in samples from the top, middle, and bottom of each dose preparation was within 91% of the target concentration. The coefficient of variation for mean values at each dose level was within 0.6%.

Concentration: Average concentrations of the test substance in samples from the 100, 500, and 2500 ppm diets were 93, 462, and 2359, respectively. Mean values for all samples were within 92% of nominal dose levels.

Conclusion:

The test material was stable in the diet for the length of time required in this study. Concentrations of the test substance in all diet levels were acceptable and were homogeneously distributed.

5. Statistics

Treated groups were compared with controls by sex. Body weight, body weight gain, hematological and biochemical data, urine volume and specific gravity, and absolute and relative organ weights were analyzed by multiple comparison tests using Dunnett's or Scheffe's method. Significance was evaluated at the $p<0.05$ and 0.01 levels. The Mann-Whitney U test was used to evaluate food consumption data and other urinalysis parameters. Fisher's exact probability test was used for clinical signs, mortality, ophthalmology, and pathology data.

C. METHODS

1. Observations

Animals were inspected at least once daily for signs of toxicity and mortality. A more detailed observation was conducted weekly.

2. Body weight

Dogs were weighed weekly, beginning during the pretest period.

3. Food consumption and food efficiency

Food consumption (g) for each animal was determined weekly. Food efficiency was not calculated.

4. Ophthalmoscopic examination

Ophthalmoscopic examinations were conducted on each dog prior to treatment and at 13 weeks of treatment. Using a direct ophthalmoscope, the eyeball, eyelid, conjunctiva, cornea, anterior chamber, pupil, iris, lens, vitreous body, and fundus were examined.

5. Blood

Blood samples were drawn from all dogs before the initiation of treatment, and after 7 and 13 weeks of treatment. Samples were collected from the cephalic vein of fasted dogs. The CHECKED (X) parameters were examined.

a. Hematology

X	Hematocrit (HCT)* (packed cell vol.)	X	Leukocyte differential count*
X	Hemoglobin (HGB)*	X	Mean corpuscular HGB (MCH)
	Methemoglobin	X	Mean corpusc. HGB conc.(MCHC)
X	Leukocyte count (WBC)*	X	Mean corpusc. volume (MCV)
X	Erythrocyte count (RBC)*		Reticulocyte count
X	Platelet count*		Heinz body count
	Blood clotting measurements*		
	(Thromboplastin time)		
	(Clotting time)		

Data taken from p. 16, MRID 45014707.

* Required for subchronic studies based on Subdivision F Guidelines

b. Clinical chemistry

	ELECTROLYTES		OTHER
X	Calcium*	X	Albumin*
X	Chloride*	X	Blood creatinine*
	Magnesium	X	Blood urea nitrogen*
X	Phosphorus*	X	Total Cholesterol
X	Potassium*	X	Globulins
X	Sodium*	X	Glucose*
		X	Total bilirubin
	ENZYMES	X	Total serum protein (TP)*
X	Alkaline phosphatase (ALK)		Osmolality
X	Serum alanine amino-transferase (ALT)*	X	Triglycerides
X	Serum aspartate amino-transferase (AST)*	X	Albumin/Globulin ratio
X	Gamma glutamyl transferase (GGT)		

Data taken from p. 17, MRID 45014707.

* Required for subchronic studies based on Subdivision F Guidelines

6. Urinalysis was conducted on all dogs prior to treatment, and at 13 weeks of treatment. Urine was collected over a 24 hour period for each animal. The CHECKED (X) parameters were examined.

x	Appearance	x	Glucose
x	Volume	x	Ketones
x	Specific Gravity	x	Blood
x	pH	x	Bilirubin
x	sediment (microscopic)	x	Urobilinogen
x	Protein		Creatinine
	Osmolality		Calcium
	Sodium		Phosphorus
	Potassium		Nitrites
	Chloride		Urea nitrogen

7. Sacrifice and pathology

All animals were anesthetized with sodium pentobarbital, exsanguinated, and necropsied. Gross morphological examinations were conducted on all dogs and major tissues and organs. The checked (XX) organs were weighed. Organs checked X were sectioned and examined microscopically for all animals.

X	DIGESTIVE SYSTEM	X	CARDIOVASC./HEMAT.	X	NEUROLOGIC
x	Salivary glands*	x	Aorta*	xx	Brain*+
x	Esophagus*	xx	Heart*+	xx	Pituitary*
x	Stomach*	x	Bone marrow*	x	Eyes
x	Intestines*	x	Lymph nodes* (mesenteric)	x	Nerve (sciatic)
x	Rectum*	xx	Spleen*+	x	Spinal Cord (cervical and lumbar)
xx	Liver*+	xx	Thymus*		**GLANDULAR**
x	Gallbladder*			xx	Adrenal gland*+
x	Pancreas*			x	Harderian glands
	RESPIRATORY		**UROGENITAL**	x	Mammary gland
		xx	Kidneys*+	xx	Parathyroids*
x	Trachea*	x	Urinary bladder*	xx	Thyroids*
x	Lung*+	xx	Testes*+		
		xx	Ovaries*+		**OTHER**
		x	Prostate	x	Skeletal muscle
		x	Uterus*	x	All gross lesions and masses*
		x	Oviduct*	x	Skin

Data taken from p. 19-22, MRID 45014707.

* Required for subchronic studies based on Subdivision F Guidelines

+ Organ weight required in subchronic and chronic studies.

II. RESULTS

A. OBSERVATIONS

1. Toxicity

The only treatment related clinical sign observed was loose stools, seen in high dose males, and mid- and high dose females (see Table 2.).

TABLE 2. Incidence of clinical signs in dogs treated with XRD-537 BE for 13 weeks.

	Dose Level (ppm)			
	0	100	500	2500
Males				
Loose stools				
weeks 1-4	0[a]	0	0	1
weeks 5-8	0	0	1	2
weeks 9-13	0	0	0	2
Females				
Loose stools				
weeks 1-4	0	0	2	2
weeks 5-8	0	0	2	2
weeks 9-13	0	0	3	1

Data taken from Tables 1-2 , pp. 59-60, MRID 45014707.
[a]Number indicates number of animals exhibiting the clinical sign.

2. Mortality

There were no deaths in treated or control animals during the study.

B. BODY WEIGHT

Group mean body weights and body weight gains are shown in Table 3. High dose males and females gained slightly less weight than controls during some weeks of the study, and 85 and 88%, respectively of what controls gained during the study overall. None of these differences were statistically significant.

TABLE 3. Selected mean body weights (kg) and body weight gains (kg) of dogs treated with XRD-537 BE for 13 weeks.				
Study week	Dose level (ppm)			
	0	100	500	2500
Body Weight, Males				
Initial	8.5	8.6	8.5	8.5
1	8.7	8.9	8.8	8.8
3	9.4	9.5	9.4	9.3
8	10.6	10.7	10.6	10.3
11	10.9	11.1	11.0	10.6
13	11.3	11.5	11.4	10.9 (96)[a]
Body Weight Gain, Males				
Week 0-1	0.3	0.3	0.3	0.3
1-5	1.4	1.5	1.5	1.3
5-10	0.9	0.9	0.9	0.7
10-13	0.5	0.5	0.5	0.4
0-13	2.8	2.9	2.9	2.4 (85)
Females, Body Weight				
Initial	8.1	8.1	8.1	8.1
1	8.5	8.4	8.5	8.5
3	9.0	8.9	9.0	8.9
8	9.8	9.7	9.9	9.7
11	10.2	10.1	10.2	10.0
13	10.6	10.5	10.6	10.3 (97)
Body Weight Gain				
Week 0-1	0.4	0.3	0.4	0.4
1-5	1.2	1.1	1.3	1.1
5-10	0.7	0.8	0.7	0.7
10-13	0.6	0.5	0.5	0.4 (67)
0-13	2.5	2.4	2.5	2.2 (88)

Data taken from Tables 5-8, pp. 63-66, MRID 45014707.
[a] Number in parenthesis represents per cent of control, calculated by reviewer.

C. FOOD CONSUMPTION and FOOD EFFICIENCY

1. Food consumption and food efficiency

Food consumption for males and females in all treated groups was comparable with controls throughout the study. Food efficiency was not calculated.

D. WATER INTAKE

Water was provided *ad libitum*, and consumption was not measured.

E. OPHTHALMOSCOPIC EXAMINATION

No visible lesions were noted in any dog at any time during the study.

F. BLOOD WORK

1. Hematology

High dose females exhibited decreases in erythrocytes and hemoglobin, $p<0.01$, and 0.05, respectively. These changes were accompanied by non-significant decreases in hematocrit, and increases in MCV. The same trends of decreased erythrocytes, hemoglobin, and hematocrit, in combination with increased MCV were also seen in the female low and mid-dose groups, although always at non-significant levels. Mean platelet count was increased in high dose females ($p<0.01$). High dose males exhibited non-significant decreases in erythrocytes, hemoglobin, and hematocrit. Platelet counts were non-significantly elevated in high dose males.

TABLE 4. Selected mean hematological values in dogs treated with XRD-537 BE for 13 weeks.

Dose Level (ppm)	0	100	500	2500
Males				
Erythrocytes x 10^6/mm^3	6.04	6.33	6.10	5.18 (86)
Hemoglobin g/dL	13.9	14.6	14.2	11.9 (86)
Hematocrit %	40.7	42.4	41.0	34.1 (84)
MCV fl	67.3	66.9	67.3	65.8 (98)
Platelets x10^3/mm^3	255	285	251	335 (131)
Females				
Erythrocytes x10^6/mm^3	6.81	6.47	6.24 (92)	5.76**(85)
Hemoglobin g/dL	15.4	14.8	14.7 (95)	13.4* (87)
Hematocrit %	44.6	43.2	42.3 (95)	39.2 (88)
MCV fl	65.6	66.8	67.8 (103)	68.2 (104)
Platelets x10^3/mm^3	265	286	274 (103)	344**(130)

Data taken from Table s 17-18, pp. 85 and 91, MRID 45014707.

* Significantly different from control group, $p<0.05$.

**Significantly different from control group, $p<0.01$.

Numbers in parenthesis represent percent of control level, calculated by reviewer.

2. Clinical chemistry

Total bilirubin was elevated ($p<0.01$) in high dose males and females. Triglycerides were decreased in high dose males ($p<0.05$), and potassium was decreased ($p<0.05$ and 0.01, respectively) in low and high dose females.

TABLE 5. Selected mean clinical chemistry parameters in dogs treated with XRD-537 BE for 13 weeks.

Dose Level (ppm)	0	100	500	2500
Males				
Total Bilirubin mg/dL	0.13	0.15	0.14	0.19**
Triglycerides mg/dL	38	42	36	29*
Females				
Total bilirubin mg/dL	0.13	0.14	0.15	0.18**
Potassium mEq/L	4.55	4.25*	4.37	4.10**

Data taken from Table 19-20, pp 96-104, MRID 45014707.
* Significantly different from control group, $p<0.05$.
**Significantly different from control group, $p<0.01$.

G. URINALYSIS

No treatment-related changes in urinalysis values were found.

H. SACRIFICE AND PATHOLOGY

1. Organ weight

Relative liver to body weight was increased ($p<0.01$) in high dose males (see Table 6). Absolute and relative thymus weights were nonsignificantly decreased in high dose males, and spleen weight was nonsignificantly increased. Absolute and relative thymus weights were decreased ($p<0.01$) in high dose females. Absolute and relative liver weights were nonsignificantly increased in high dose females.

TABLE 6. Selected mean absolute and relative[a] organ weights (g) among dogs fed XRD-537 BE for 13 weeks.

Observation	Dietary concentration (ppm)			
	0	100	500	2500
Males				
Liver				
absolute	291	299	288	339 (116)
relative	2.59	2.61	2.54	3.11**(120)
Thymus				
absolute	11.5	10.2	11.3	7.9 (69)
relative	0.10	0.09	0.10	0.07 (70)
Spleen				
absolute	27.5	30.0	29.0	46.7 (170)
relative	0.25	0.26	0.25	0.41 (164)
Females				
Liver				
absolute	258	266	299	282 (109)
relative	2.44	2.55	2.82	2.73 (112)
Thymus				
absolute	12.3	11.6	11.1 (90)	5.4** (44)
relative	0.12	0.11	0.11 (92)	0.06** (50)
Spleen				
absolute	25.3	26.0	29.4	26.1 (103)
relative	0.24	0.25	0.28	0.25 (104)

Data taken from Tables 23-24, pp. 107-110, MRID 45014707.
[a] Mean organ weight relative to mean body weight
** Significantly different from control, $p < 0.01$.
Numbers in parenthesis indicate percent of control vales, calculated by reviewer.

2. Gross pathology

All high dose males exhibited a brownish thymus and distended gallbladder at necropsy (see Table 7). Three of four high dose females exhibited a grossly atrophied thymus, and 3/4 had a distended gallbladder. Distended gallbladders were also seen in 3/4 mid-dose females. Other gross changes occurred equally in controls or in single animals.

TABLE 7. Gross findings in dogs treated with XRD-537 BE for 13 weeks.				
Dose Group(ppm)	**0**	**100**	**500**	**2500**
Finding	**Males**			
Thymus brownish	0	0	0	4*
Gallbladder distended	0	0	0	4*
	Females			
Thymus atrophy	0	0	0	3
Gallbladder distended	0	0	3	3

Data taken from Tables 21-22, pp.105-106, MRID 45014707.
*Significantly different from the control, $p<0.05$.

3. Microscopic pathology

Increased cytoplasmic eosinophilia of hepatocytes was found in 4/4 high dose males and females (see Table 8). Thymic atrophy was seen in 3/4 high dose males, and 1/4 controls. Hyaline droplet degeneration of proximal tubular cells was present in 2/4 and 3/4 high dose males and females, respectively. Both males and females exhibited splenic gamna gandy nodules (associated with splenic enlargement, e.g. congestive and siderotic splenomegaly) in some animals in control and treated groups. All high dose females exhibited histologic thymus atrophy. Pale colored colloid and follicular epithelial hypertrophy were present in thyroids of 2/4 high dose females.

TABLE 8. Microscopic changes in dogs treated with XRD-537 BE for 13 weeks.				
Dose (ppm)	**0**	**100**	**500**	**2500**
Males				
Kidney-hyaline droplet degeneration of proximal tubular cells	0	0	0	2
Spleen, Gamna-Gandy nodule	2	2	3	1
Liver-increased cytoplasmic eosinophilia of hepatocytes	0	0	0	4*
Thymus Atrophy	1	0	0	3
Females				
Thyroid Follicular epithelia hypertrophy, Pale-colored colloid	0 0	0 0	0 0	2 2
Kidney-hyaline droplet degeneration of proximal tubular cells	0	0	0	3
Liver-increased cytoplasmic eosinophilia of hepatocytes	0	0	2	4*
Spleen, Gamna-Gandy nodule	1	1	2	2
Thymus Atrophy	0	0	0	4*

Data taken from Tables 25-26, pp. 111-114, MRID 45014707.
*Significantly different from control, $p<0.05$.

III. DISCUSSION

A. DISCUSSION

No treatment-related mortality occurred during the study. The only clinical sign that could be attributed to the test substance was loose stools, occurring in mid-dose and high dose males and females. All treated groups had mean body weights within 4% of control levels. Low and mid-dose males and females had body weight gains comparable to controls. High dose males and females had cumulative body weight gains 85 and 88% respectively, of controls for the 13 week study. These differences were not considered statistically significant, although they may be treatment related. Food consumption among all treated groups was comparable with controls throughout the study. No ophthalmic lesions were found in any dog at any time during the study.

High dose females exhibited decreases in erythrocytes and hemoglobin, $p<0.01$ and 0.05, respectively, and non-significant decreases in hematocrit, and increases in MCV. The same trends of decreased erythrocytes, hemoglobin, and hematocrit, in combination with increased MCV were also seen in low and mid-dose females. High dose males also had decreases in erythrocytes, hemoglobin and hematocrit. These results are consistent with a mild treatment-related anemia. Mean platelet count was significantly increased in high dose females, and non-significantly increased in high dose males, but these changes are quite small considering the normal variability in platelet counts in dogs. All these hematologic variations are mild and not toxicologically meaningful.

Although total bilirubin was significantly elevated ($p<0.01$) in high dose males and females, this finding is of questionable biological significance, since the highest levels attained were within the normal range for dogs. Triglycerides were significantly decreased in high dose males ($p<0.05$), and potassium was significantly decreased ($p<0.05$ and 0.01, respectively) in low and high dose females. Although possibly treatment related, these findings are not toxicologically meaningful. No treatment-related changes in urinalysis values were found.

Relative liver weight was increased ($p<0.01$) in high dose males, and non-significantly increased in mid- and high dose females. These increased liver weights correlate with the histologic finding of increased cytoplasmic eosinophilia of hepatocytes in all high dose males and females, indicating a possible hypertrophic response. Absolute and relative thymus weights were nonsignificantly decreased in high dose males, and decreased ($p<0.01$) in high dose females. The reduced thymus weights in concert with findings of both gross and histologic thymic atrophy in these groups are consistent with the anemia indicated above, and perhaps an impaired immune function. Increased absolute and relative splenic weights in high dose males could also be considered related to the hematological changes, however, these values were greatly skewed by dramatic increases in a single animal, reducing their significance.

The hyaline droplet degeneration of proximal tubular cells found in kidneys of 2/4 and 3/4 high dose males and females, respectively, indictates nephritis, possibly treatment

related. Follicular epithelial hypertrophy and pale colloid found in thyroids of 2/4 high dose females are of undetermined significance. Distended gallbladders are common in laboratory dogs because of the need to use low fat feed.

Under the conditions of this study, the LOAEL for the systemic toxicity of XRD-537 BE in beagle dogs is 2500 ppm (75.2, and 79.4 mg/kg/day for males and females, respectively) based on brown and/or atrophied thymuses, and decreased thymus weight. The NOAEL is 500 ppm (14.7 and 15.6 mg/kg/day in males and females, respectively).

This subchronic toxicity study is classified as **Acceptable/Guideline.** This study does satisfy the Subdivision F requirements for a 90-day oral toxicity study in non-rodents [OPPTS 870.3150 (§82-1)].

B. STUDY DEFICIENCIES

None found.

APPENDIX

Palatability study of XRD-537nBu in beagle dogs.

Objective: The objective of this study was to evaluate potential toxicity and palatability of XRD-537nBU in Beagle dogs.

Citation: Mizell, M., K.T. Haut, and J.W. Crissman. (1990). XRD-537nBu: Palatability and four-week dietary probe study in beagle dogs. Toxicology Research Laboratory, Health and Environmental Sciences, Dow Chemical Co., Midland, MI 48674. Laboratory Project Study ID DR-0298-8876-004, September 11, 1990. MRID 45000410. Unpublished.

Dosing: Dosages of 250, 500, or 1000 mg/kg/day were administered to single dogs in feed for two weeks, during the palatability portion of the study. Dosages of 0, 35, 100 or 350 mg/kg/day were administered to two dogs/sex/group in feed for four weeks, during the toxicity range-finding portion of the study.

Methods: Animal care and maintenance, assignment to study groups, preparation of doses and animal observations were similar to those described in the main study. The toxicity range-finding portion of the study, originally planned to last two weeks, was extended an additional two weeks since all animals appeared clinically normal at the end of the first two weeks. Statistical evaluation of data was not performed due to the small number of animals used.

Results:

1. Palatability study

   No clinical signs were observed during the study. The dogs given 500 or 1,000 mg/kg/day lost 12% of their body weight during the study. The dog given 250 mg/kg/day lost only 2% of its body weight. The high dose dog had dramatically reduced feed consumption, suggesting decreased palatability of the treated diet at this dose level. Dogs treated with 250 or 500 mg/kg/day had no gross lesions. The dog treated with 1000 mg/kg/day had a distended gallbladder, which the authors dismissed as a result of poor feed consumption.

2. Toxicity study

   No treatment related clinical signs were observed during the study. High dose males and females had reduced food consumption, and lost 17 and 26% of their body weight, respectively. One high dose female essentially rejected the diet. High dose males and females had increased relative (to body weight) liver and kidney weights. Pathologic effects in high dose animals included moderate to severe thymus atrophy, bile duct hyperplasia, loss of hepatocellular glycogen, increased hepatocyte necrosis, and diffuse subacute inflammation in the liver. Multifocal vasculitis and thrombosis of the kidney was seen in both sexes and high dose males had moderately to severely impaired spermatogenesis. Slight gross and histologic thymic atrophy were seen in two mid- and one low dose dogs, and one mid-dose female had the renal vascular lesion to a slight degree.

Conclusion: Based on the results of this study, the authors concluded that a NOAEL for XRD-537nBu in Beagle dogs was not established. However, because of the minimal and equivocal effects of the low dose, it was suggested that 35 mg/kg/day was very near to the NOAEL. Based on the results of this study, levels were set for the subchronic oral study of XRD-537nBu in Beagle dogs at 0, 100, 500, or 2500 ppm, equivalent to 2.9, 14.7, and 75.2 mg/kg/day in males, and 3.2, 15.6, and 79.4 mg/kg/day in females.

DATA EVALUATION RECORD

CYHALOFOP BUTYL
(DE-537 N-BUTYL ESTER)

STUDY TYPE: REPEATED DOSE DERMAL – RAT [OPPTS 870. 3200 (§82-2)]
MRID 45000415

Prepared for

Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
1921 Jefferson Davis Highway
Arlington, VA 22202

Prepared by

Chemical Hazard Evaluation Group
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Disclaimer

This review may have been altered subsequent to the contractor's signatures above.

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CYHALOFOP BUTYL

Repeated Dose Dermal Study [OPPTS 870.3200 (§82-2)]

EPA Reviewer: J. Whalan
Registration Action Branch 2 (7509C)
EPA Work Assignment Manager: S. Williams-Foy, D.V.M.
Registration Action Branch 2 (7509C)
TXR No: 0050348

Date: 5-9-01

Date 5/31/24

DATA EVALUATION RECORD

STUDY TYPE: Repeated Dose Dermal – Rat [OPPTS 870.3200 (§82-2)]

DP BARCODE: D268553
P.C.CODE.: 082583

SUBMISSION CODE: not given
TOX. CHEM. NO: not given

TEST MATERIAL (PURITY): DE-537 N-Butyl Ester (97.1%)

SYNONYMS: Cyhalofop-butyl, XRD-537 nBU, XRD-537 BE, butyl (2R)-2-[4-(4-cyano-2-fluorophenoxy) phenoxy] propanoate

CITATION: Crissman, J.W. and Zablotny, C.L. (1999) DE-537 N-Butyl Ester: 4-Week Dermal Toxicity and 2-Week Recovery Study in Fischer 344 Rats. Health & Environmental Research Laboratories, The Dow Chemical Company, Midland, Michigan 48674. Laboratory Study No. 981127, January 19, 1999. MRID 45000415. Unpublished.

SPONSOR: Dow AgroSciences (DAS) LLC, 9330 Zionsville Rd., Indianapolis, IN 46268.

EXECUTIVE SUMMARY: In a repeated dose dermal toxicity study (MRID 45000415), groups of 5 male and 5 female Fischer 344 rats received applications of 0, 10, 100, 1000 mg/kg/day DE-537 n-butyl ester (97.1%, Lot No. DECO-26-42T) in aqueous 0.5% methylcellulose, 6 hours/day, 5 days/week for 4 weeks. Additional recovery groups of 5 control and 5 high-dose rats/sex were held an additional 2 weeks in order to evaluate the potential reversibility of any effects noted during the exposure period.

There were no treatment-related deaths, clinical signs, dermal effects, opthalmological effects, or gross or microscopic pathology. No treatment-related body weight or food consumption effects were observed.

An increase in prothrombin time from 14.8 to 16.1 seconds (8.8%) was observed from high-dose males compared to controls at the end of the 4-week treatment period. Serum cholesterol was decreased 24% in high-dose males and 15% in high-dose females compared to controls after 4-weeks of treatment. Albumin was increased ($p<0.05$) and globulin was decreased ($p<0.05$) in high-dose males and females combined when compared to controls. Absolute liver weights were increased ($p<0.05$) in high-dose males (15.3%) and females (5.1%) compared to controls at the end of the 4-week treatment period. The relative liver weight of only high-dose males was increased ($p<0.05$) 11.2% over controls at the end of the 4-week exposure period. Following, the 2-week recovery period, mean liver weights of high-dose males and females were not different than those of controls. When considered together, the reversibility of the clinical chemistry and

liver weight effects are considered biological markers of exposure and are not considered toxicologically significant.

The systemic NOAEL for DE 537 n-butyl ester in male and female rats is ≥the limit dose of 1000 mg/kg/day and the systemic LOAEL is not identified. The dermal NOAEL is ≥the limit dose of 1000 mg/kg/day and the dermal LOAEL is not identified.

This study is classified as **Acceptable/Guideline** and does satisfy the guideline requirements for a repeated-dose dermal study [OPPTS 870.3200 (§82-2)] in rats.

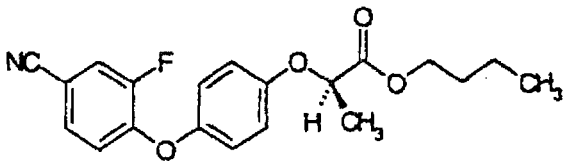
COMPLIANCE: Signed and dated Quality Assurance, Data Confidentiality, and Good Laboratory Practice Statements were present. No Flagging statement was present.

MATERIALS AND METHODS

A. MATERIALS

1. Test material: DE-537 n-butyl ester (97.1%)

Synonyms: Cyhalofop-butyl, XRD-537 nBU, XRD-537 BE
Description: off-white powder
Lot/Batch #: DECO-26-42T
Purity/Stability: 97.1%/DE-537 n-butyl ester was shown to be stable in aqueous methylcellulose for at least 17 days
Structure:



2. Vehicle and/or positive control

Vehicle: aqueous 0.5% methylcellulose
Positive control: none

3. Test animals

Species: Rat
Strain: Fischer 344
Age and weight at study initiation: males: approx. 8 weeks, 167.1-196.3 g; females: approx. 8 weeks, 115.6- 135.2
Source: Charles River Laboratories, Inc., Raleigh, NC
Housing: 1/cage in suspended, wire mesh stainless steel cages
Diet: Purina Certified Rodent Lab Diet #5002, *ad libitum*
Water: tap water, *ad libitum*
Environmental conditions:
Temperature: 19- 25°C

Humidity: 40-70%
Air changes: 12-15/hour
Photoperiod: 12 hour light/12 hour dark cycle
Acclimation period: at least 7 days

B. STUDY DESIGN

1. In life dates

Start: August 20, 1998 End: September 17, 1998 (main study);
October 1, 1998 (recovery)

2. Animal assignment

Rats were distributed within the experimental groups (Table 1) using a computer-generated randomization based on body weight.

TABLE 1. Experimental design

Group	Dose Level (mg/kg/day)	No. of Animals (main study)		No. of Animals (recovery)	
		Male	Female	Male	Female
Control	0	5	5	5	5
Low-dose	10	5	5	-	-
Mid-dose	100	5	5	-	-
High-dose	1000	5	5	5	5

3. Dose selection rationale

The high dose level is the limit dose of 1000 mg/kg/day. The lower doses were expected to provide dose-response data and a NOAEL.

4. Test substance preparation and analysis

DE-537 n-butyl ester was applied as a suspension in an aqueous vehicle of 0.5% methyl cellulose (METHOCEL) at a dose volume of 2.5 mL/kg. Dose amounts were calculated for each rat based on weekly body weights. Dosing suspensions were mixed once prior to study initiation and approximately 2 weeks later. Concentrations of all dose level preparations were assessed using HPLC at the start of the study and was verified during the use of the second mix. Homogeneity of the low- and high-dose preparations was determined for the initial mix. Stability of DE-537 n-butyl ester in methocel was established by reanalyzing all the dose preparations after 17 days.

Results –

Homogeneity analysis – The 10 mg/kg and 1000 mg/kg dosing suspensions were homogeneously mixed at study initiation, with percent relative standard deviations of 2.00% and 1.03%, respectively.

Stability analysis – Dosing suspensions were shown to be stable for 17 days, which encompasses the period of use.

Concentration analysis – The mean achieved concentrations for the dose preparations analyzed were 94-113% of the nominal concentrations.

Homogeneity, stability, and concentration are considered acceptable for the purposes of this study.

5. Dose application

An area of approximately 10% of the total body surface on the back of each rat was clipped free of hair prior to study initiation and as necessary thereafter. The dosing preparation was applied directly to the skin at a volume of 2.5 mL/kg. The application site was covered with a porous gauze patch and non-absorbent cotton held in place by an elastic bandage. After 6 hours, the dressings were removed and the treated sites wiped with water dampened towels. Animals were treated 6 hours/day, 5 days/week over a 4-week period (20 total applications). Control animals were treated the same, except only aqueous methylcellulose was applied.

6. Statistics

Means and standard deviations were calculated for all continuous data. All parameters examined statistically were first tested for equality of variance using Bartlett's test. In-life body weights were analyzed using a repeated measures (RM) analysis of variance (ANOVA) for time, sex, and dose. Final body weight, absolute and relative organ weight (excluding ovaries, uterus, epididymides and testes), hematologic parameters (excluding WBC differential and RBC) and clinical chemistry parameters were analyzed with a two-way ANOVA, and comparisons of individual dose groups to the control was made with Dunnett's test when a dose effect existed at alpha = 0.05. Results for ovaries, uterus, testes, and epididymides weights were analyzed using one-way ANOVA. Food consumption was analyzed by Bartlett's test for equality of variances. Descriptive statistics only were reported for body weight gain, erythrocyte indices, and differential WBC counts.

C. METHODS

1. Observations

Animals were examined for mortality and signs of toxicity twice daily. A detailed clinical observation (DCO) was implemented on day 7 and included careful physical examination with evaluation of sensory reactivity to stimuli. DCOs were conducted

weekly on all animals for the remainder of the 4-week dosing period. DCOs were not conducted during the 2-week recovery period since no treatment-related effects were noted during the 4-week dosing period. Open field observations and sensory evaluations were made in a 50 cm X 50 cm clear plastic box.

2. Body weight

Animals were weighed prestudy and weekly throughout the study and during the 2-week recovery period.

3. Food consumption

Individual food consumption was calculated weekly during the study.

4. Food efficiency

Food efficiency was not calculated by the study authors.

5. Ophthalmoscopic examination

Ophthalmoscopic examination was performed on all rats (after pupil dilation with 0.5% tropicamide) on days 5 and 26. No examinations were performed during the recovery period.

6. Blood samples were obtained from the orbital sinus of all fasted animals, anesthetized with methoxyflurane, at the scheduled 4-week termination. The CHECKED (X) parameters were examined.

a. Hematology

X		X	
x	Hematocrit (HCT)	x	Leukocyte differential count
x	Hemoglobin (HGB)		Mean corpuscular HGB (MCH)
x	Leukocyte count (WBC)		Mean corpusc. HGB conc.(MCHC)
x	Erythrocyte count (RBC)		Mean corpusc. volume (MCV)
x	Platelet count		Reticulocyte count
	Blood clotting measurements		
	(Thromboplastin time)		
	(Clotting time)		
x	(Prothrombin time)		
x	Erythrocyte morphology		

b. Clinical chemistry

X	ELECTROLYTES	X	OTHER
x	Calcium	x	Albumin
x	Chloride	x	Blood creatinine
	Magnesium	x	Blood urea nitrogen
x	Phosphorus	x	Total Cholesterol
x	Potassium		Albumin/Globulin ratio
x	Sodium	x	Glucose
		x	Total bilirubin
	ENZYMES	x	Total serum protein
x	Alkaline phosphatase(ALK)		(TP)
	Cholinesterase(ChE) (plasma & red blood cell)		Triglycerides
x	Creatine kinase		Serum protein electrophoresis
	Lactic acid dehydrogenase(LDH)		
x	Serum alanine amino-transferase (also SGPT)		
x	Serum aspartate amino-transferase (also SGOT)		
	Gamma glutamyltransferase(GGT)		
	Glutamate dehydrogenase		
	Sorbitol dehydrogenase		

7. Urinalysis

Urinalysis was not required and was not performed.

8. Sacrifice and pathology

Fasted rats were anesthetized with methoxyflurane vapors and euthanized by decapitation. All animals were given post mortem examinations. The CHECKED (X) tissues were preserved in 10% neutral buffered formalin. The tissues were embedded in paraffin, sectioned at 6 µm, and stained with hematoxylin and eosin. All high-dose and control tissues were examined as well as skin from low- and mid-dose animals. The (XX) tissues were also weighed.

X	DIGESTIVE SYSTEM	X	CARDIOVASC./HEMAT.	X	NEUROLOGIC
x	Tongue	x	Aorta	xx	Brain
x	Salivary glands	xx	Heart	x	Periph. nerve
x	Esophagus	x	Bone marrow	x	Spinal cord
x	Stomach	x	Lymph nodes	x	Pituitary
x	Duodenum	xx	Spleen	x	Eyes (optic n.)
x	Jejunum	xx	Thymus		
x	Ileum				GLANDULAR
x	Cecum		UROGENITAL	xx	Adrenal gland
x	Colon	xx	Kidneys[a]	x	Lacrimal gland
x	Rectum	x	Urinary bladder	x	Mammary gland
xx	Liver[ab]	xx	Testes[a]	x	Parathyroids
	Gall bladder	xx	Epididymides	x	Thyroids
x	Pancreas	x	Prostate		
		x	Seminal vesicle		OTHER
	RESPIRATORY	xx	Ovaries	x	Bone
x	Trachea	xx	Uterus	x	Skeletal muscle
x	Lung[a]	x	Cervix	x	Skin (treated anduntreated)[a]
x	Nose	x	Vagina	x	All gross lesions and masses[a]
	Pharynx				
x	Larynx				

[a]Required for subchronic studies based on OPPTS 870.3200.
[b]The tissues were analyzed for main study animals as indicated in the table. Additionally, only the liver was weighed for the recovery animals since this organ was the only one showing an effect at the end of the 4-week treatment period. No microscopic analysis was performed on the recovery animals.

II. RESULTS

A. OBSERVATIONS

No animals died during the study, and there were no treatment-related clinical observations or dermal effects.

B. BODY WEIGHT

There were no biologically significant, treatment-related effects noted. There was a 2.4% increase ($p<0.05$) in body weight of high-dose males on study day 28. The difference did not persist during the recovery period.

C. FOOD CONSUMPTION AND EFFICIENCY

1. Food consumption

No treatment-related food consumption effects were observed.

2. Food efficiency

Food efficiency was not calculated.

D. OPHTHALMOSCOPIC EXAMINATION

No treatment-related ophthalmoscopic effects were noted.

E. BLOODWORK

An increase in prothrombin time from 14.8 to 16.1 seconds (8.8%) was observed in high-dose males compared to controls at the end of the 4-week treatment period. Serum cholesterol was decreased 24% in high-dose males and 15% in high-dose females

compared to controls after 4-weeks of treatment. Albumin was increased ($p<0.05$) and globulin was decreased ($p<0.05$) in high-dose males and females combined when compared to controls. No effect on total protein was noted. Blood urea nitrogen was decreased ($p<0.05$) in mid-dose males and females combined; however, in the absence of an effect at the high-dose and since the measured values were within the historical control range for this laboratory, the urea nitrogen effect is not considered treatment-related.

F. URINALYSIS

Urinalysis was not required and was not performed.

G. SACRIFICE AND PATHOLOGY

1. Organ weight

Absolute liver weights were increased ($p<0.05$) in high-dose males (15.3% increase compared to controls) and females (5.1% increase compared to controls) at the end of the 4-week treatment period. The relative liver weight of only high-dose males was increased ($p<0.05$) 11.2% over controls at the end of the 4-week exposure period.

Following, the 2-week recovery period, mean liver weights of high-dose males and females were not different than those of controls.

2. Gross pathology

No treatment-related effects were noted.

3. Microscopic pathology

No treatment-related effects were noted.

III. DISCUSSION

A. There was no test substance-related mortality during the study. There were no treatment-related signs of systemic toxicity, dermal irritation, effects on body weight, food consumption, opthalmoscopic effects, or gross or microscopic pathology.

An 8.8% increase in prothrombin time was observed in high-dose males compared to controls at the end of the 4-week treatment period. Serum cholesterol was decreased 24% in high-dose males and 15% in high-dose females compared to controls after 4-weeks of treatment. Albumin was increased ($p<0.05$) and globulin was decreased ($p<0.05$) in high-dose males and females combined when compared to controls. None of these effects are clinically relevant and all are within historical limits.

Absolute liver weights were increased ($p<0.05$) in high-dose males (15.3%) and females (5.1%) and relative liver weight of high-dose males was increased 11.2% compared to controls at the end of the 4-week treatment period. Following, the 2-week recovery period, mean liver weights of high-dose males and females were not different than those of controls. When considered together, the reversibility of the clinical chemistry and liver weight effects are indicative of mild changes in liver metabolism. The effects are considered biological markers of exposure and are not considered toxicologically significant.

The systemic NOAEL for DE 537 n-butyl ester in male and female rats is ≥the limit dose of 1000 mg/kg/day and the systemic LOAEL is not identified. The dermal NOAEL is ≥the limit dose of 1000 mg/kg/day and the dermal LOAEL is not identified.

B. STUDY DEFICIENCIES

None identified.

This study is classified as **Acceptable/Guideline** and does satisfy the guideline requirements for a repeated-dose dermal study [OPPTS 870.3200 (§82-2)] in rats.

DATA EVALUATION RECORD

CYHALOFOP BUTYL
[XRD 537 BE]

STUDY TYPE: DEVELOPMENTAL TOXICITY – RAT [OPPTS: 870.3700 (§ 83-3a)]
MRID 45014709

Prepared for

Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
1921 Jefferson Davis Highway
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Prepared by

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Task Order No. 01-81Z

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Disclaimer

This review may have been altered subsequent to the contractors' signatures above.

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CYHALOFOP BUTYL **Developmental Toxicity Study [870.3700 (§83-3)]**

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TXR No.: 0050348

Date: 6-5-02

Date: 7/9/02

DATA EVALUATION RECORD

STUDY TYPE: Developmental Toxicity - Rat [OPPTS 870.3700 (§83-3)]

DP BARCODE: D268553
P.C. CODE: 082583

SUBMISSION CODE: S
TOX. CHEM. NO.: none

TEST MATERIAL (PURITY): XRD-537 BE (Cyhalofop butyl, 97.1% a.i.)

SYNONYMS: R-(+)-n-Butyl-2-(4-(2-fluoro-4-cyanophenoxy)phenoxy)propanoate; XDE-537; XRD-537; XRD-537 nBu; XDE-537 nBu; XRD-537 n Butyl Ester; DEH-112

CITATIONS: Hatakenaka, N. (1992) Teratogenicity study in rats with XRD-537 BE. Kodaira Laboratories, The Institute of Environmental Toxicology, Suzuki-cho 2-772, Kodaira-shi, Tokyo 187, Japan. Laboratory Study ID. IET 90-0173, December 21, 1992. MRID 45014709. Unpublished.

SPONSOR: DowElanco Japan Ltd., Seavans North 2-1, Shibaura 1-chome, Minato-ku, Tokyo 105, Japan.

EXECUTIVE SUMMARY: In a developmental toxicity study (MRID 45014709), 24 mated Specific Pathogen Free Crj:CD(SD) rats per group were administered XRD-537 BE (Lot No.: DECO-26-42T [AGR 295713]; purity: 97.1%) by gavage in 1 % aqueous sodium carboxymethylcellulose at doses of 0, 25, 250, or 1000 mg/kg/day on gestation days (GDs) 6-15, inclusive. On GD 20, all surviving dams were sacrificed and necropsied, and all fetuses were weighed, sexed, and examined for external malformations/variations. Approximately one-half of the fetuses from each litter were examined for visceral alterations by fresh dissection, and the remaining fetuses were subjected to skeletal examination.

At the 1000 mg/kg/day treatment level, the liver to body weight ratio and the liver to adjusted body weight ratio were both increased (106-107% of controls; $p<0.01$), and there were slight, non-statistical increases in the mean absolute liver weights of all treated groups; however, these increases can be attributed to enzyme induction as an adaptive response to a xenobiotic agent rather than a treatment-related adverse effect. There were no treatment-related effects observed at 25 and 250 mg/kg/day.

The maternal toxicity NOAEL is 1000 mg/kg/day (limit dose).

There were no treatment-related differences in intrauterine parameters, including mean numbers of corpora lutea and implantation sites, pre- and post-implantation loss, viable litter size, fetal sex ratios, or fetal weights. The overall incidence rates for litters containing fetuses with external, visceral, or skeletal malformations in the 0, 25, 250, and 1000 mg/kg/day groups were 4/22, 3/23, 1/23, and 5/24, respectively, and there were no significant increases in litter or fetal incidences of any individual malformations or variations for any treated group.

The developmental toxicity NOAEL is greater than or equal to 1000 mg/kg/day (limit dose).

This study is classified as **Acceptable/Guideline** and satisfies the requirements for a developmental toxicity study [OPPTS: 870.3700 (§83-3)] in rats. However, the study report did not describe the storage conditions of the dosing formulations.

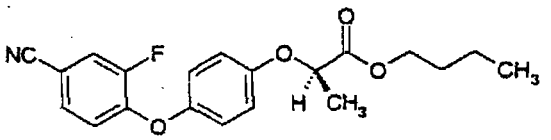
COMPLIANCE: Signed and dated Data Confidentiality, Good Laboratory Practice, Quality Assurance, and Flagging statements were included.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: XRD-537 BE

   Description: Off-white powder
   Lot No.: DECO-26-42T [AGR 295713]
   Purity: 97.1%
   Contaminants: 0.4% S isomer of the active ingredient
   Stability of compound: "Stable in a dark and cold environment"
   CAS No.: 122008-85-9
   Structure:



2. Vehicle and/or positive control

   The vehicle control article was a 1 % aqueous solution of sodium carboxymethylcellulose in purified water (Wako Pure Chemical Industries, Ltd.; Lot no. CTJ 0627). No positive control was used in this study.

3. Test animals

   Species: Rat
   Strain: Specific Pathogen Free Crj:CD(SD)
   Age and weight at study initiation: 13 weeks; 223-299 g on GD 0
   Source: Charles River Japan, Inc.

Housing: individually in suspended aluminum cages with wire-mesh floors and fronts, 260 mm x 400 mm x 240 mm
Diet: Pulverized feed (NMF, Oriental Yeast Co., Ltd.) was available to females *ad libitum.*
Water: Local tap water was available *ad libitum.*
Environmental conditions:
Temperature: 24±2°C
Humidity: 55±10%
Air changes: 12/hour
Photoperiod: 12 hr light/12 hr dark
Acclimation period: 7 days prior to study initiation

B. PROCEDURES AND STUDY DESIGN

This study was designed to assess the maternal and developmental toxicity potential of XRD-537 BE when administered by gavage to rats on GD 6-15, inclusive.

1. In life dates

Start: March 16, 1992; end: May 10, 1992

2. Mating

Vaginal smears from the females were collected and examined. Females in proestrus were paired with males of the same strain and source overnight at a ratio of one female to one male. Successful mating was assessed by observation of a vaginal plug or spermatozoa in a vaginal smear, and the day on which a vaginal plug and/or spermatozoa were observed was designated as gestation day (GD) 0.

3. Animal assignment and dose selection are presented in Table 1. Animals were randomized and assigned to groups in such a way as to equalize group means and standard deviations of body weights.

TABLE 1: Animal assignment

Test Group	Dosage Level (mg/kg/day)	Number Assigned
1. Control	0	24
2. Low-Dose	25	24
3. Mid-Dose	250	24
4. High-Dose	1000	24

Data taken from text, p. 6-7, MRID 45014709.

4. Dose selection rationale

Doses were selected based on the results of a preliminary study in the rat (IET 90-0172). A summary of the range-finding study was included in the main report; however, no data were provided. Doses of 0, 10, 100, 300, and 1000 mg/kg/day were administered to seven rats per group by gavage on GD 6-15. No maternal mortality or fetotoxicity was observed at dose levels up to 1000 mg/kg/day. At the 1000 mg/kg/day dose level, maternal body weight gains and food consumption were slightly decreased during the early dosing interval, and no adverse effects were observed at dose levels of 300 mg/kg/day or less. Based on these results, the dose levels chosen for the main study were 0, 25, 250, and 1000 mg/kg/day.

5. Dosing

All doses were administered in a volume of 10 mL/kg of body weight, based on the most recently recorded body weight.

6. Dose solution preparation and analysis

Test article formulations were prepared every six days during dosing. The report did not give details of the method of dosing formulation preparation other than stating that "the test substance was suspended in purified water with the aid of 1% CMC." The dosing formulations were stirred constantly with a magnetic stirrer during the dosing procedure in order to maintain homogeneity. The report also did not mention the storage conditions of the dosing formulations. Prior to the initiation of dosing, duplicate samples from the top, middle, and bottom of the low- and high-dose formulations were analyzed for homogeneity, and duplicate samples of all dosing solutions from all three mixes were collected for concentration analysis prior to use. Stability analysis was conducted at an earlier date, in conjunction with the preliminary study (IET 90-0172), and these results were summarized in the report. The analytical method used was high performance liquid chromatography. A separate study (MRID 45000528) was provided in which suspensions of radiolabelled and/or non-radiolabelled XRD-537 BE in 0.5% CMC either alone or with 2% Tween 80 were analyzed for homogeneity and/or stability.

Results -

Concentration analysis: Absence of test article was confirmed in the vehicle. Mean concentrations of the low-, mid-, and high-dose formulations were 96-102%, 98-102%, and 93-96%, respectively, of nominal. All of the individual measured concentrations were within 8% of nominal.

Homogeneity analysis: The overall mean measured concentrations of the low- and high-dose formulations were 100.5% and 101.3% of nominal with coefficients of variation of 0.3 and 1.6%, respectively, and the measured values for the individual samples ranged from 98 to 104% of nominal.

Stability analysis: The results of the preliminary study (IET 90-0172) indicated that the enantiomer ratios (R/S) in the low- and high-dose solutions were 99.08/0.92 and 99.01/0.99, respectively, at day 0 and 99.00/1.0 and 99.01/0.99, respectively, after refrigerated storage for 7 days. Stability of solutions of the test material in 0.5% CMC with 2% Tween 80 for up to 21 days of storage at 4 °C was also confirmed in MRID 45000528.

The results from the analyses of the dosing formulations showed that the test article could be adequately mixed in the vehicle and was stable for the duration of use assuming that the formulations were stored under refrigerated conditions. Therefore, doses to the animals were considered adquate.

C. OBSERVATIONS

1. Maternal observations and evaluations

The animals were observed for clinical signs and mortality once a day during GD 0-5 and twice a day during GD 6-20. Body weights were recorded on GDs 0, 3, 6-15, and 20, and food consumption was recorded for the following intervals: GD 0-3, 3-6, 6-9, 9-12, 12-15, and 15-20. Dams were sacrificed on GD 20 by an overdose of ether anesthesia and subjected to gross necropsy. The reproductive tract was excised, and the gravid uterine weight as well as numbers of corpora lutea, implantations, and dead or live fetuses, and resorptions were recorded. Resorptions or dead fetuses were classified as implantation sites, placental remnants, or macerated fetuses. While these categories were not further defined in the study report, the reviewer is interpreting them as roughly corresponding to early resorptions, late resorptions, and late fetal deaths, respectively, although "macerated fetuses" could also include some late resorptions, and "placental remnants" could also include some early resorptions. Uteri without gross evidence of implantation were checked for evidence of early implantation loss by the Salewski method. In addition, maternal livers and kidneys were weighed and retained with the ovaries in 10% neutral buffered formalin.

2. Fetal evaluations

Fetal and placental weights were recorded, then fetuses were sexed and subjected to external examination, including examination of the eyes by removing the palpebral skin. Approximately one-half of the fetuses from each litter were examined for visceral alterations by the Stuckhardt and Poppe fresh dissection technique then retained in 10% neutral buffered formalin, along with the placentas. The remaining fetuses were fixed in 70% alcohol, eviscerated, stained with Alizarin Red S, then subjected to skeletal examination and retained in 70% glycerine.

D. DATA ANALYSIS

1. Statistical analysis

Maternal body weights and body weight changes, maternal food consumption, gravid uterine weights, fetal and placental weights, organ weights, and numbers of corpora lutea, total implantations, and viable fetuses were subjected to statistical analysis as follows. Bartlett's test was used to evaluate the homogeneity of the variances. Data with homogeneous variances were subjected to a one-way parametric analysis of variance followed by Dunnett's t-test or Scheffe's multiple comparison test if significant. Data with heterogeneous variances were subjected to the Kruskal-Wallis test followed by a Dunnett-type mean rank test or a Scheffe-type mean rank test if significant. Incidence data such as clinical signs, gross necropsy findings, fetal sex ratios, and fetal and litter incidences of malformations and variations were analyzed using Fisher's exact probability test. Litter proportions of resorptions and fetal deaths were analyzed using the Mann-Whitney U test. A minimum significance level of $p<0.05$ was used for all analyses.

2. Historical control data were provided for the fetal and litter incidences of three particular visceral variations (thymic remnant in the neck, dilatation of the renal pelvis and/or ureter, and left umbilical artery) in Crj:CD (SD) rats. Information regarding the dates of the twelve studies, the source of the animals, and the vehicle(s) and route(s) of administration was not provided.

II. RESULTS

A. MATERNAL TOXICITY

1. Mortality and clinical signs

There were no deaths during the study. At the 1000 mg/kg/day treatment level, two animals exhibited soiled fur in the external genital area on GDs 11 and 9-12, and a third animal exhibited soiled fur in the external genital area on GD 9 and exhibited red vaginal discharge on GD 14. At the 250 mg/kg/day treatment level, one animal exhibited red vaginal discharge on GD 18. Other clinical signs such as a subcutaneous mass and hair loss on various body surfaces were not considered treatment related.

2. Body weight

Selected maternal body weight data are given in Table 2. There were no significant differences between mean absolute body weights and adjusted body weights of the control and treated groups. The study authors calculated mean body weight gains as being cumulative with respect to the GD 0 body weight. The cumulative body weight

gains by the 1000 mg/kg/day group were statistically decreased for the GD 0-7, 0-9, and 0-10 intervals (83, 72, and 83% of controls, respectively; $p<0.05$).

TABLE 2: Selected mean maternal body weights and body weight changes during gestation (g)				
GD	**0 mg/kg/day**	**25 mg/kg/day**	**250 mg/kg/day**	**1000 mg/kg/day**
Absolute body weights				
0	261	259	260	261
6	294	291	296	294
9	303	302	305	295
12	319	316	320	311
15	339	339	343	332
20	416	416	422	410
Adjusted body weight[a]	332	333	337	329
Body Weight Changes				
0-7	35	35	36	29*
0-15	78	79	82	71
0-20	155	157	162	150

Data taken from Tables 3 and 4, pp. 24 and 25, respectivley, MRID 45014709.
[a]Adjusted body weight = terminal body weight minus gravid uterine weight.

3. Food consumption

Selected maternal food consumption data are given in Table 3. Mean food consumption by the 1000 mg/kg/day group was statistically decreased during the GD 6-9 and 9-12 intervals (82 and 91% of controls, respectively; $p<0.01$).

TABLE 3: Selected mean maternal food consumption during gestation (g/animal/day)				
GD	**0 mg/kg/day**	**25 mg/kg/day**	**250 mg/kg/day**	**1000 mg/kg/day**
0-3	20	20	20	20
3-6	22	22	23	22
6-9	22	22	22	18** (82)[a]
9-12	23	23	23	21** (91)
12-15	23	23	23	21
15-20	25	25	26	26

Data taken from Table 5, p. 26, MRID 45014709.
[a] Number in parentheses is per cent of control; calculated by reviewer.
Significantly different from control; **$p<0.01$.

4. Gross pathology

Abnormal necropsy findings included a subcutaneous mass in one animal from the control group, hair loss on various body surfaces in one animal from the 25 mg/kg/day group and three animals from the 1000 mg/kg/day group, and unilateral or bilateral renal pelvic dilatation in two animals from the 1000 mg/kg/day group. None of these findings were considered treatment related.

7. Organ weight data

At the highest dose level, the liver to body weight ratio and the liver to adjusted body weight ratio were both increased (107 and 106% of controls, respectively; $p<0.01$). Mean absolute liver weights of all treated groups were slightly increased (102, 104, and 105% of controls for the low-, mid-, and high-dose groups, respectively; n.s.).

6. Cesarean section data

Data collected at the scheduled cesarean section are summarized in Table 4. There were no total litter resorptions or late fetal deaths (macerated fetuses). There were no significant differences between the treated and control groups in mean numbers of corpora lutea and implantation sites, pre- and post-implantation losses, resorptions (implantations, placental remnants, or macerated fetuses), viable litter size, or fetal weights. At the 250 mg/kg/day dose level, the fetal sex ratio was statistically increased (53.4 vs. 46.3% male in the control group; $p<0.05$); this is not considered a treatment-related effect as a similar difference was not observed in the 1000 mg/kg/day group.

The female from the 1000 mg/kg/day group that exhibited red vaginal discharge on GD 14 had 16 corpora lutea, 15 live fetuses, and 1 late resorption (placental remnant). The female from the 250 mg/kg/day group that exhibited red vaginal discharge on GD 18 had 18 corpora lutea, two early resorptions (implantation sites), and one late resorption (placental remnant).

TABLE 4: Cesarean section observations				
Observation	**0 mg/kg/day**	**25 mg/kg/day**	**250 mg/kg/day**	**1000 mg/kg/day**
No. Animals Assigned	24	24	24	24
No. Animals Pregnant	22	23	23	24
Pregnancy Rate[a] (%)	92	96	96	100
Maternal Mortality	0	0	0	0
Delivered Early/Aborted	0	0	0	0
Pregnant at scheduled necropsy	22	23	23	24
Total Corpora Lutea	373	379	385	400
Corpora Lutea/dam	17.0	16.5	16.7	16.7
Total Implantations	354	369	376	385
Implantations/Dam	16.1	16.0	16.3	16.0
Preimplantation Loss[b] (%)	5.1	2.6	2.3	3.8
Postimplantation Loss (mean %)	4.5	5.2	5.8	5.0
Total Live Fetuses	339	350	354	365
Viable Fetuses/Litter	15.4	15.2	15.4	15.2
Mean Fetal Weight (mg)				
Males	3461	3553	3596	3397
Females	3370	3357	3434	3231
Sex Ratio (% Male)	46.3	47.7	53.4*	49.0
Total fetal resorptions and deaths per litter	0.68	0.83	0.96	0.83
"Macerated fetuses[c]" per litter	0	0	0	0
"Placental remnants[c]" per litter	0.09	0	0.22	0.13
"Implantation sites[c]" per litter	0.59	0.83	0.74	0.71
Dams with all resorptions	0	0	0	0

Data taken from Tables 1 and 8 and Appendices 25-28, pp 20, 29, and 62-65, respectively, MRID 45014709.
[a]Calculated by reviewer as Pregnancy rate = (number of animals pregnant/number of animals mated) x 100.
[b]Calculated by reviewer as Preimplantation Loss = [(Total Corpora Lutea-Total Implantations)/ Total Corpora Lutea]×100.
[c]These terms were not further defined in the study report. The reviewer is interpreting them as follows: "macerated fetuses" roughly corresponds to late fetal deaths, although some late resorptions could also be included; placental remnants" roughly corresponds to late resorptions, although some early resorptions could also be included; and implantation sites" roughly corresponds to early resorptions, excluding any that are included as "placental remnants."
Significantly different from control; *p<0.05, **p<0.01

B. DEVELOPMENTAL TOXICITY

The numbers of fetuses (litters) examined in the 0, 25, 250, and 1000 mg/kg/day groups were 339 (22), 350 (23), 354 (23), and 365 (24), respectively. The overall incidence rates for litters containing fetuses with external, visceral, and/or skeletal malformations in the 0, 25, 250, and 1000 mg/kg/day groups were 4/22, 3/23, 1/23, and 5/24, respectively. Selected fetal morphological data are given in Table 5.

1. External examination

One fetus from the 25 mg/kg/day group had ectopia cordis, and one fetus from the 1000 mg/kg/day group had microphthalmia. No external malformations were observed in the 0 or 250 mg/kg/day groups, and no external developmental variations were observed in any group.

2. Visceral examination

An aberrant right subclavian artery was observed in one fetus from the 25 mg/kg/day group, and there were no visceral malformations observed in the 0, 250, and 1000 mg/kg/day groups. Visceral variations included thymic remnant in the neck, left umbilical artery, and dilatation of the renal pelvis and/or ureter. The fetal incidence of dilatation of the renal pelvis and/or ureter was statistically increased at the 1000 mg/kg/day dose level (6.2 vs 0.6% of fetuses for controls; $p<0.01$), and the litter incidence of this finding was non-statistically increased (16.7 vs. 4.5% for controls); however, both the fetal and litter incidences fell within historical control ranges (0.0-6.2% and 0.0-25.0%, respectively). Statistical decreases in fetal incidences of the visceral variation thymic remnant in neck and total visceral variations at the 25 mg/kg/day dose level were not considered treatment related.

3. Skeletal examination

The following skeletal malformations were observed: splitting of ossification centers of the thoracic vertebral bodies in three fetuses (three litters), one fetus, one fetus, and three fetuses (three litters) from the 0, 25, 250, and 1000 mg/kg/day groups, respectively; absence of the left cervical vertebral arch in one fetus from the control group; absence of ossification centers of the thoracic vertebral bodies in one fetus from the 250 mg/kg/day group; and asymmetry of the sternebrae with sterno-costal joint displacement in one fetus from each of the 25 and 1000 mg/kg/day groups. Skeletal variations included cervical ribs, wavy ribs, shortening or absence of the 13th ribs, lumbar ribs, 25 presacral vertebrae, sacralization of the lumbar vertebrae, and asymmetry of the sternebrae. There were no differences between the treated and control groups in fetal or litter incidences of these findings, which occurred at single or low incidences and are therefore considered to be spontaneous, rather than an adverse effect of treatment.

TABLE 5: Fetal malformations and variations [Number of litters (fetuses)]				
Observation	**0 mg/kg/day**	**25 mg/kg/day**	**250 mg/kg/day**	**1000 mg/kg/day**
Malformations				
Ectopia cordis	0/22 (0/399)	1/23 (1/350)	0/23 (0/354)	0/24 (0/365)
Microphthalmia	0/22 (0/399)	0/23 (0/350)	0/23 (0/354)	1/24 (1/365)
Aberrant right subclavian artery	0/22 (0/163)	1/23 (1/169)	0/23 (0/171)	0/24 (0/178)
Absence of the left cervical vertebral arch	1/22 (1/176)	0/23 (0/181)	0/23 (0/183)	0/24 (0/187)
Splitting of ossification centers of the thoracic vertebral bodies	3/22 (3/176)	1/23 (1/181)	1/23 (1/183)	3/24 (3/187)
Absence of ossification centers of the thoracic vertebral bodies	0/22 (0/176)	0/23 (0/181)	1/23 (1/183)	0/24 (0/187)
Asymmetry of the sternebrae with sterno-costal joint displacement	0/22 (0/176)	1/23 (1/181)	0/23 (0/183)	1/24 (1/187)
Variations				
Thymic remnant in the neck	7/22 (18/163)	7/23 (7/169*)	10/23 (13/171)	7/24 (13/178)
Dilatation of the renal pelvis and/or ureter	1/22 (1/163)	2/23 (2/169)	5/23 (5/171)	4/24 (11/178**)
Left umbilical artery	2/22 (2/163)	2/23 (2/169)	0/23 (0/171)	1/24 (1/178)
Cervical ribs	1/22 (1/176)	3/23 (3/181)	3/23 (5/183)	1/24 (1/187)
Wavy ribs	1/22 (1/176)	1/23 (1/181)	0/23 (0/183)	0/24 (0/187)
Shortening or absence of the 13th ribs	2/22 (2/176)	1/23 (1/181)	2/23 (2/183)	1/24 (2/187)
Lumbar ribs	1/22 (1/176)	1/23 (1/181)	2/23 (2/183)	3/24 (4/187)
25 presacral vertebrae	1/22 (1/176)	1/23 (1/181)	0/23 (0/183)	1/24 (1/187)
Sacralization of the lumbar vertebrae	0/22 (0/176)	0/23 (0/181)	0/23 (0/183)	2/24 (4/187)
Asymmetry of the sternebrae	2/22 (4/176)	3/23 (3/181)	1/23 (1/183)	4/24 (4/187)

Data taken from Table 11, p. 32, MRID 45014709.
Significantly different from control; * $p<0.05$, ** $p<0.01$

III. DISCUSSION

A. INVESTIGATORS' CONCLUSIONS

The study author concluded that XRD-537 BE resulted in maternal toxicity at 1000 mg/kg/day as evidenced by decreased (cumulative) maternal weight gain during GD 0-7, 0-9, and 0-10, decreased food consumption during GD 6-12, and increased relative liver weights. There were no treatment-related adverse effects on intrauterine parameters, including implants and live fetuses, fetal resorptions and deaths, viable litter size, fetal sex ratios, mean fetal body weights, gravid uterine weights, and placental weights. There were also no treatment-related effects on fetal structural development. The study author concluded that the maternal toxicity NOAEL and LOAEL were 250 and 1000 mg/kg/day, respectively, and that 1000 mg/kg/day was a developmental toxicity NOAEL.

B. REVIEWER'S DISCUSSION

1. MATERNAL TOXICITY

At the 1000 mg/kg/day dose level, maternal body weights were 2-3% lower, the adjusted body weight was 1% lower, and food consumption was 9-18% lower than the controls during the dosing interval. These changes are too small to be considered toxicologically significant.

The results of a special study on hepatocellular proliferation in rats (MRID 45000414), indicated that the hepatic effects of the test substance in rats were consistent with an adaptive response by the liver to a xenobiotic agent, rather than an adverse effect of treatment. The increased relative liver weights in the current study are, therefore, also attributed to enzyme induction as an adaptive response rather than a treatment-related adverse effect.

The maternal toxicity NOAEL is 1000 mg/kg/day (limit dose).

2. DEVELOPMENTAL TOXICITY

a. Deaths/resorptions

Maternal treatment with XRD-537 BE did not result in an increase in fetal deaths or resorptions.

b. Altered growth

No evidence of altered growth was seen in this study.

c. Developmental variations

Treatment with the test article did not result in an increased incidence of fetal variations.

d. Malformations

Treatment with the test article did not result in an increased incidence of fetal malformations.

The developmental toxicity NOAEL is greater than or equal to 1000 mg/kg/day (limit dose).

C. STUDY DEFICIENCIES

Randomization procedures and sacrifice order were not provided. The study report did not include a copy of the study protocol and any amendments. The previously mentioned, minor deficiencies do not compromise the integrity of the study because they do not prevent assessment of NOAELs and LOAELs. However, the study report did not describe the storage conditions of the dosing formulations.

D. CORE CLASSIFICATION

This study is classified as **Acceptable/Guideline** and satisfies the requirements for a developmental toxicity study [OPPTS: 870.3700 (§83-3a)] in rats.

DATA EVALUATION RECORD

CYHALOFOP BUTYL
[XRD 537 BE]

STUDY TYPE: DEVELOPMENTAL TOXICITY – RABBIT [OPPTS: 870.3700 (§ 83-3)]
MRID 45014710

Prepared for

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Office of Pesticide Programs
U.S. Environmental Protection Agency
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Prepared by

Chemical Hazard Evaluation Group
Toxicology and Risk Analysis Section
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Oak Ridge, TN 37831
Task Order No. 01-81AA

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Disclaimer

This review may have been altered subsequent to the contractors' signatures above.

Oak Ridge National Laboratory, managed and operated by UT-Battelle, LLC, for the U.S. Department of Energy under contract number DE-AC05-00OR22725.

CYHALOFOP BUTYL **Developmental Toxicity Study [870.3700 (§83-3)]**

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Date: 7/9/02

DATA EVALUATION RECORD

STUDY TYPE: Developmental Toxicity - Rabbit [OPPTS 870.3700 (§83-3)]

DP BARCODE: D268553
P.C. CODE: 082583

SUBMISSION CODE: none
TOX. CHEM. NO.: none

TEST MATERIAL (PURITY): XRD-537 BE (Cyhalofop butyl, 97.1% a.i.)

SYNONYMS: R-(+)-n-Butyl-2-(4-(2-fluoro-4-cyanophenoxy)phenoxy)propanoate; XDE-537 BE; XDE-537; XRD-537; XRD-537 nBu; XDE-537 nBu; XRD-537 n Butyl Ester; DEH-112

CITATIONS: Aoyama, H. (1994) A teratogenicity study in rabbits with XRD 537 BE. The Institute of Environmental Toxicology, Suzuki-cho 2-772, Kodaira-shi, Tokyo 187, Japan. Laboratory Study ID. GHF-P-1391, March 8, 1994. MRID 45014710. Unpublished.

E.W. Carney, *et al.* (2001) XDE-537 Teratogenicity Study in Rabbits: Reanalysis of Fetal anomaly Data. Toxicology & Environmental Research and Consulting, The Dow Chemical Company, Midland, Michigan. Report ID DR-0287-1467-012, May 21, 2001, MRID 45413901

SPONSOR: Nichimen Corporation, 11-1, Nihonbashi 3-chome, Chuo-ku, Tokyo 103, Japan; Dow Chemical Japan Ltd., Seavans North 2-1, Shibaura 1-chome, Minato-ku, Tokyo 105, Japan.

EXECUTIVE SUMMARY: In a developmental toxicity study (MRID 45014710 and 45413901), 18 inseminated specific pathogen free Japanese White (Kbl:JW) rabbits per group were administered XRD-537 BE (Lot No.: AGR 295713; purity: 97.1%) by gavage in 1% aqueous carboxymethylcellulose at doses of 0, 40, 200, or 1000 mg/kg/day on gestation days (GDs) 6-18, inclusive, with the day following the day of artificial insemination designated as GD 0. On GD 27, all surviving does were sacrificed and necropsied, and all fetuses were weighed and subjected to external examination, then sexed internally and examined viscerally by fresh dissection, followed by evisceration and processing for skeletal examination.

The evaluation of this study is complicated by a high incidence of maternal mortality. In the 1000 mg/kg/day group, seven deaths occurred on GDs 15-19 following hematuria on the day of death and, in most cases, one and/or two days prior to death; one dam was killed *in extremis* on

GD 13 due to exhibiting "ventral posture;" and an additional dam died on GD 25 without exhibiting any abnormal clinical signs. In the 200 mg/kg/day group, one dam died on GD 21 after exhibiting loose stool and/or soiled fur in the lower abdominal/external genital region, hematuria, and feces with yellowish white, viscous material; and another dam aborted and was sacrificed on GD 25, following observations of hematuria and loose stool. Hematuria was also observed in one dam each in the control and 200 mg/kg/day groups during the pre-dosing interval, in another control dam on GD 24, and in one dam in the 200 mg/kg/day group on GD 18. The number of animals exhibiting hematuria during the dosing interval was significantly increased ($p<0.01$) at the 1000 mg/kg/day dose level. Considering the pattern of occurrence, what was reported as hematuria was probably concentrated, blood-colored urine which can be attributed to dehydration, rather than to disease or the test article.

Absolute body weights and body weight gains were similar in all groups through GD 18, but increased in a dose-related manner after dosing ceased (GDs 18-27) except for the controls which lost body weight. Adjusted body weight also increased in a dose-related manner. Food consumption mirrored body weight gain, with the most notable anomaly being a significant decrease in mean food consumption in the control dams due to anorexia in 5 of 18 dams and reduced food consumption in several other dams.

The predominant necropsy findings from the animals that died after exhibiting hematuria included "cloudy" or dark colored kidneys, whitish membranous material on the gastric mucosa or white spots in the stomach, urinary bladder distended with red or brown urine, and scanty gastrointestinal contents. Necropsy findings from the surviving animals also included "dark" or cloudy colored kidneys in three high-dose animals, and "cloudy" colored kidneys in one mid-dose animal. The significance of these findings is unknown.

The maternal LOAEL is 200 mg/kg/day based on maternal death. The maternal NOAEL is 40 mg/kg/day.

There were no treatment-related effects on intrauterine parameters. The overall incidence rates for litters containing fetuses with external, visceral, and/or skeletal malformations in the 0, 40, 200, and 1000 mg/kg/day groups were 2/17 (11.8%), 5/18 (27.8%), 7/16 (43.8%), and 0/9, respectively.

At the 200 mg/kg/day dose level, but not at the 1000 mg/kg/day dose level, the litter incidence of total malformations was significantly greater than controls ($p<0.05$) and exceeded the historical control range (11.8-31.3%), and the litter incidence of skeletal malformations was also increased (6/16 vs. 1/17 for controls; $p<0.05$) although there were no increased incidences of any individual malformations. These increases were most likely due to maternal stress rather than test article toxicity.

The developmental NOAEL is ≥1000 mg/kg/day (limit dose).

This study is classified as **Acceptable/Guideline** and does satisfy the requirements for a developmental toxicity study [OPPTS: 870.3700 (83-3b)] in rabbits.

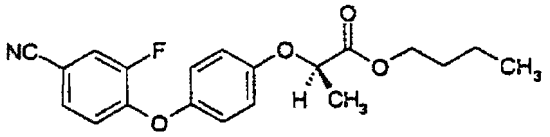
COMPLIANCE: Signed and dated Data Confidentiality, Good Laboratory Practice, Quality Assurance, and Flagging statements were included.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: XRD-537 BE

Description: Off-white powder
Lot No.: AGR 295713
Purity: 97.1%
Stability of compound: "Stable in a dark and cold environment"
CAS No.: 122008-85-9
Structure:



2. Vehicle and/or positive control

The vehicle control article was a 1 % aqueous solution of carboxymethylcellulose in purified water (CMC, Kanto Chemical Co., Inc., Lot no. 207N1441). No positive control was used in this study.

3. Test animals

Species: Rabbit
Strain: Specific pathogen free Japanese White (Kbl:JW)
Age and weight at study initiation: approximately 18 weeks; 3484-4402 g. on GD 0
Source: Ina Research Laboratory, KITAYAMA LABES Co., Ltd.
Housing: individually in suspended aluminum cages with wire-mesh floors, 350 mm x 480 mm x 330 mm
Diet: Solid feed (GC4, Oriental Yeast Co., Ltd.) was available *ad libitum*.
Water: Well water passed through precipitating and sedimentary procedures and sterilized with hypochlorous acid and ultraviolet light exposure was available *ad libitum*.
Environmental conditions:
Temperature: 22±2°C
Humidity: 55±10%
Air changes: approximately 15/hour
Photoperiod: 12 hr light/12 hr dark
Acclimation period: 17 days prior to study initiation

B. PROCEDURES AND STUDY DESIGN

This study was designed to assess the maternal and developmental toxicity potential of XRD-537 BE when administered by gavage to rabbits on GD 6-18, inclusive.

1. In life dates

Start: November 9, 1992; end: December 11, 1992

2. Artificial insemination

Females were inseminated using pooled semen collected from 3 resident males of the same strain as the females. Diluted semen was used to inseminate twelve or sixteen does. Each doe received an intravenous injection of human chorionic gonadotropin immediately following insemination. The day after the day of artificial insemination was designated as gestation day (GD) 0.

3. Animal assignment and dose selection are presented in Table 1. Animals were randomized and assigned to groups in such a way as to equalize group means and standard deviations of body weights.

TABLE 1: Animal assignment

Test Group	Dosage Level (mg/kg/day)	Number Assigned
1. Control	0	18
2. Low-Dose	40	18
3. Mid-Dose	200	18
4. High-Dose	1000	18

Data taken from text, p. 6, MRID 45014710.

4. Dose selection rationale

Doses were selected based on the results of preliminary studies in the rabbit. The report stated that there were no overt toxic effects on maternal rabbits or their fetuses at dose levels up to 1000 mg/kg/day. No other data from the preliminary studies were provided.

5. Dosing

All doses were administered in a volume of 5 mL/kg of body weight, based on the most recently recorded body weight.

6. Dose solution preparation and analysis

Test article formulations were prepared weekly during the study. The report did not give details of the method of dose solution preparation other than stating that "the test

substance was suspended in purified water with the aid of 1% CMC." The report also did not mention the storage conditions of the dosing formulations. Duplicate samples of all dosing solutions from all three mixes were collected for concentration analysis prior to use. The analytical method used was high performance liquid chromatography. Stability analysis was conducted at an earlier date, in conjunction with a preliminary study (IET 90-0174), and these results were summarized in the report, but no data were included. Homogeneity analysis of dosing solutions of the test article in 1% CMC was conducted in conjunction with a developmental toxicity study in rats (MRID 45014709); however, the method of dose solution preparation was not described for this study either and may have been different from that used in the current study. A separate stability and homogeneity study (MRID 45000528) was provided in which suspensions of radiolabelled and/or non-radiolabelled XRD-537 BE in 0.5% CMC either alone or with 2% Tween 80 were analyzed for homogeneity of radioactivity and/or stability.

Results –

Concentration analysis: Absence of test article was confirmed in the vehicle. Mean concentrations of the low-, mid-, and high-dose formulations were 96-100%, 98-101%, and 97-100%, respectively, of nominal. All of the individual measured concentrations were within 5% of nominal.

Homogeneity analysis: Homogeneity was confirmed in MRID Nos. 45014709 and 45000528. However, in MRID 45000528 it was noted that the solutions became non-homogenous when allowed to stand for 5-30 minutes after preparation.

Stability analysis: The study author stated that results of the preliminary study (IET 90-0174) indicated that the test substance was stable in a 1% CMC solution for at least 10 days. The storage conditions used in the preliminary study (IET 90-0174) were not described, and none of the data from the preliminary study were provided. Stability of solutions of the test material in 0.5% CMC with 2% Tween 80 for up to 21 days of storage at 4°C was confirmed in MRID 45000528.

The analytical results from the concentration analyses of the dosing formulations were satisfactory. However, the study did not include adequate information about the storage conditions of the dosing formulations, and there was no mention made of stirring the dosing formulations during dosing to maintain homogeneity. Nevertheless, the rabbits likely received the expected dosages.

C. OBSERVATIONS

1. Maternal observations and evaluations

The animals were observed at least once a day from GD 0 through GD 27 for clinical signs and mortality. Body weights were recorded on GDs 0, 3, 6-18, 21, 24, and 27, and food consumption was recorded on alternate days during GDs 0-26 and on GD 27. Dams were sacrificed on GD 27 by intravenous injection of sodium pento-

barbital and subjected to gross necropsy. The reproductive tract was excised, and the number of corpora lutea on each ovary was recorded. The gravid uterine weight as well as numbers of viable and nonviable fetuses, resorptions, and implantations were recorded. Resorptions or dead fetuses were classified as implantation sites, placental remnants, or macerated fetuses. While these categories were not further defined in the study report, the reviewer is interpreting them as roughly corresponding to early resorptions, late resorptions, and late fetal deaths, respectively, although "macerated fetuses" could also include some late resorptions, and "placental remnants" could also include some early resorptions. Uteri without gross evidence of implantation were checked for evidence of early implantation loss by the Salewski method. In addition, the liver, spleen, kidneys, and ovaries were retained in 10% neutral buffered formalin. All animals that aborted, died, or were sacrificed moribund were subjected to gross necropsy.

2. Fetal evaluations

Fetal and placental weights were recorded, then fetuses were euthanized and subjected to external examination, including examination of the eyes by removing the palpebral skin. Fetuses were sexed internally and examined for visceral alterations by the Stuckhardt and Poppe fresh dissection technique. Fetal thoracic and abdominal organs were retained in 10% neutral buffered formalin, along with the placentas. All fetal skeletons were stained with Alizarin Red S and cleared in 70% glycerin, than subjected to skeletal examination.

D. DATA ANALYSIS

1. Statistical analysis

Maternal body weights and body weight changes, maternal food consumption, gravid uterine weights, fetal and placental weights, and numbers of corpora lutea, total implantations, and viable fetuses were subjected to statistical analysis as follows. Bartlett's test was used to evaluate the homogeneity of variances. Data with homogeneous variances were subjected to a one-way parametric analysis of variance followed by Dunnett's t-test or Scheffe's multiple comparison test if significant. Data with heterogeneous variances were subjected to the Kruskal-Wallis test followed by a Dunnett-type mean rank test or a Scheffe-type mean rank test if significant. Incidence data such as clinical signs, gross necropsy findings, fetal sex ratios, and fetal and litter incidences of malformations and variations were analyzed using Fisher's exact probability test. Litter proportions of resorptions and fetal deaths were analyzed using the Mann-Whitney U test. A minimum significance level of $p<0.05$ was used for all analyses.

The sponsor, the Dow Chemical Company, submitted a supplemental study report (MRID 45413901; May 21, 2001) which revealed the statistical methods used to evaluate incidences of lumbar ribs were not entirely appropriate. In the initial study report, it states that a Fisher's exact probability test was used for data on the

incidences of maternal rabbits having fetuses with malformations and variations (i.e., a litter based statistic), which is an acceptable method. However, the investigators also ran an additional Fisher's test to analyze the percentage of fetuses with specific malformations and variations, which is not appropriate. That the investigators ran separate Fisher's tests for both the percentage of affected litters and the percentage of affected fetuses was corroborated by the appearance of Table 10 in the report, in which statistical significance for fetal and litter incidences are denoted separately.

The lumbar rib data were reanalyzed using the Censored Wilcoxon test, which is a litter-based analysis, but which uses the fetal incidence data as a secondary ranking factor when the litter is totally unaffected, and combined the probabilities from both analyses. Using an appropriate litter-based statistical method that is ideally suited for developmental toxicity incidence data, no evidence of an increase in the incidence of lumbar ribs was found. The results of this reanalysis are reflected in this DER.

2. Historical control data were not provided although the historical control ranges for preimplantation loss, overall litter incidence of malformations, and the fetal incidence of the skeletal variation lumbar ribs were mentioned in the results or discussion sections of the study report. Information regarding the number of studies included, dates of the studies, source of the animals, and the vehicle(s) and route(s) of administration was not provided.

II. RESULTS

A. MATERNAL TOXICITY

1. Mortality and clinical signs

There were seven deaths in the 1000 mg/kg/day group on GDs 15-19 following the observation of hematuria on the day of death and, in most cases, one and/or two days prior to death. The animal that died on GD 15 also exhibited hypoactivity on GD 14. An additional animal from the 1000 mg/kg/day group died on GD 25 without exhibiting any abnormal clinical signs, and one animal was killed *in extremis* on GD 13 due to exhibiting "ventral posture." In the 200 mg/kg/day group, one animal died on GD 21 after exhibiting loose stool and/or soiled fur in the lower abdominal/external genital region on GDs 14-21, hematuria on GDs 19-21, and feces with yellowish white, viscous material on GD 19. One animal from the 200 mg/kg/day group aborted and was sacrificed on GD 25, following observations of hematuria on GDs 18 and 22 and loose stool on GDs 14-19 and 21-24.

Clinical signs observed from animals that survived to termination included the following: one female in each of the control and 200 mg/kg/day groups exhibited hematuria during the pre-dosing interval, on GDs 1 and 5, respectively; and hematuria was also observed from a different control group animal on GD 24 and from one animal from the 200 mg/kg/day group on GD 18.

At the 1000 mg/kg/day dose level, the number of animals exhibiting hematuria during the dosing interval (GD 7-19) was significantly greater than controls (0/17, 0/18, 2/18, and 7/18 animals from the 0, 40, 200, and 1000 mg/kg/day groups, respectively; $p<0.01$). The number of animals exhibiting hematuria at any time during the study was 2/17, 0/18, 4/18, and 7/18 (n.s.) animals from the 0, 40, 200, and 1000 mg/kg/day groups, respectively.

2. Body weight

Selected maternal body weight data are given in Table 2. Absolute body weights and body weight gains were similar in all groups through GD 18, but increased in a dose-related manner after dosing ceased (GDs 18-27) except for the controls which lost body weight. Adjusted body weight also increased in a dose-related manner.

TABLE 2: Selected mean maternal body weights and body weight changes during gestation (g)

GD	0 mg/kg/day	40 mg/kg/day	200 mg/kg/day	1000 mg/kg/day
Absolute body weights				
0	3850	3868	3868	3865
6	3966	3979	3986	3969
12	4016	4030	4049	3991
18	4054	4102	4067	4068
24	4087	4208	4146	4269
27	4041	4209	4228	4279
Adjusted body weight[a]	3591	3778	3822	3929
Body weight changes				
0-6	116	111	118	104
0-18	204	234	199	203
18-27	-13	107	161	211
0-27	191	341	360	414

Data taken from Tables 3 and 4, pp. 24-25, and 26, respectively, MRID 45014710.
[a]Adjusted body weight = terminal body weight minus gravid uterine weight.

3. Food consumption

Selected maternal food consumption data are given in Table 3. There were non-statistical decreases in mean food consumption of the 1000 mg/kg/day group for all measuring intervals between GDs 8 and 18 (71-91% of controls). These decreases do not corresponded to the mean body weight gains which were similar in all groups during the dosing interval. Mean food consumption by the 1000 mg/kg/day group was statistically increased at all measuring intervals between GDs 20 and 27 (150-246% of controls; $p<0.05$ or 0.01); mean food consumption by the 200 mg/kg/day

group was statistically increased during GDs 24-27 (198-202% of controls; $p<0.05$); and mean food consumption by the 40 mg/kg/day group was statistically increased at the GD 22-24 interval (170% of controls; $p<0.05$). Thus, food consumption mirrored body weight gain, with the most notable anomaly being a significant decrease in mean food consumption in the control dams due to anorexia in 5 of 18 dams and reduced food consumption in several other dams.

TABLE 3: Selected mean maternal food consumption during gestation (g/animal/day)

GD	0 mg/kg/day	40 mg/kg/day	200 mg/kg/day	1000 mg/kg/day
0-2	181	172	177	175
2-4	183	176	183	182
4-6	177	166	178	178
6-8	164	152	169	152
8-10	170	153	161	145 (85)[a]
10-12	145	147	142	125 (86)
12-14	128	134	125	117 (91)
14-16	136	125	127	97 (71)
16-18	144	143	136	113 (78)
18-20	129	158	136	158 (122)
20-22	105	141	133	158* (150)
22-24	71	121* (170)	106	130* (183)
24-26	54	101	107* (198)	133** (246)
26-27	47	87	95* (202)	112* (238)

Data taken from Table 5, p. 27, MRID 45014710.
[a] Number in parentheses is per cent of control; calculated by reviewer.
Significantly different from control; *$p<0.05$, **$p<0.01$.

4. Gross pathology

Necropsy findings from the seven high-dose animals that were found dead on GDs 15-19 after exhibiting hematuria included the following: "cloudy" colored kidneys in four animals, "dark" colored kidneys in two animals, and enlarged kidneys with red spots in one animal; whitish membranous material on the gastric mucosa in three animals; urinary bladder distended with red or brown urine in two animals; tricho-bezoars (hairballs) in two animals, one of which also had white spots in the stomach; scanty gastrointestinal contents in three animals, one of which also had red contents in the small intestine; and ascites in the animal that had enlarged kidneys with red spots. Necropsy findings from the high-dose animal that was killed *in extremis* on GD 13 included "cloudy" colored kidneys and black, watery stomach contents. Necropsy findings from the high-dose animal that was found dead on GD 25, without

any prior abnormal clinical signs included watery stomach contents and an umbilical hernia of small intestine. Necropsy findings from the mid-dose animal that was found dead on GD 21 after exhibiting hematuria and loose stool included "cloudy" colored kidneys and a trichobezoar in the stomach with whitish membranous material on the gastric mucosa. Necropsy findings from the mid-dose animal that was sacrificed on GD 25 following loose stool, hematuria, and abortion included a trichobezoar in the stomach and gaseous distension of the large intestine.

Necropsy findings from the nine surviving high-dose animals included "dark" colored kidneys in one animal and "cloudy" colored kidneys in two animals. Necropsy findings from the 16 surviving mid-dose animals included "cloudy" colored kidneys in one animal, trichobezoars in three animals, watery and scanty large intestinal contents in one animal, and gaseous distension of the large intestine in one animal. One low-dose animals had a trichobezoar. Necropsy findings from control group animals included trichobezoars in six animals, one of which also had gaseous distension of the large intestines, and one of which also had a pale liver.

5. Cesarean section data

Data collected at the scheduled cesarean section are summarized in Table 4. There were no total litter resorptions. There were no significant differences between the treated and control groups in the mean numbers of corpora lutea/dam, late fetal deaths (macerated fetuses), late resorptions (placental remnants), early resorptions (implantation sites), post-implantation losses, or fetal sex ratios. The mean number of implantations per dam in the 1000 mg/kg/day dose group was significantly decreased as compared to controls (6.0 vs. 9.5 for controls; $p<0.05$), and corresponded to an increase in the mean pre-implantation loss (34.6 vs. 12.0% for controls). Since dosing was initiated after implantation was believed to have occurred, the decreased mean number of implantations and increased pre-implantation loss are probably not an adverse effect of treatment and may just be an artifact of the small sample size.

The mean number of viable fetuses per litter was significantly decreased at the 1000 mg/kg/day dose level (5.6 vs. 9.1 for controls; $p<0.01$); however, this was probably due to the increased pre-implantation loss and decreased number of implantations rather than being an adverse effect of treatment. The significantly ($p<0.05$) increased mean fetal weights for both male and female fetuses at the 1000 mg/kg/day dose level were most likely due to the smaller liter size of this group.

TABLE 4: Cesarean section observations				
Observation	**0 mg/kg/day**	**40 mg/kg/day**	**200 mg/kg/day**	**1000 mg/kg/day**
No. Animals Assigned	18	18	18	18
No. Animals Pregnant	17	18	18	18
Pregnancy Rate[a] (%)	94	100	100	100
Maternal Mortality	0	0	1	9[b]
Delivered Early/Aborted	0	0	1	0
Pregnant at scheduled necropsy	17	18	16	9
Total Corpora Lutea[c]	183	190	185	82
Corpora Lutea/dam	10.8	10.6	11.6	9.1
Total Implantations[c]	161	150	153	54
Implantations/Dam	9.5	8.3	9.6	6.0*
Preimplantation Loss[d] (mean %)	12.0	21.1	17.5	34.6
Postimplantation Loss (mean %)	3.5	6.9	13.0	9.9
Total Live Fetuses	155	138	132	50
Viable Fetuses/Litter	9.1	7.7	8.3	5.6**
Mean Fetal Weight (g)				
Males	35.4	40.1	37.4	44.2*
Females	34.6	37.6	35.4	43.2*
Sex Ratio (% Male)	51.0	53.6	54.5	50.0
Total fetal resorptions and deaths per litter	0.35	0.67	1.31	0.44
"Macerated fetuses[e]" per litter	0.06	0.44	0.75	0.33
"Placental remnants[e]" per litter	0.06	0.00	0.31	0.00
"Implantation sites[e]" per litter	0.24	0.22	0.25	0.11
Dams with all resorptions	0	0	0	0

Data taken from Tables 1 and 7 and Appendices 21-24, pp 20, 29, and 59-62, respectively, MRID 45014710.

[a] Calculated by reviewer as Pregnancy rate = (number of animals pregnant/number of animals mated) x 100.

[b] Includes one animal killed *in extremis*.

[c] Calculated by reviewer using individual data.

[d] Calculated by reviewer from individual data as group means for the following:
Preimplantation Loss = [(Corpora Lutea-Implantations)/Corpora Lutea] x 100.

[e] These terms were not further defined in the study report. The reviewer is interpreting them as follows: "macerated fetuses" roughly corresponds to late fetal deaths, although some late resorptions could also be included; "placental remnants" roughly corresponds to late resorptions, although some early resorptions could also be included; and "implantation sites" roughly corresponds to early resorptions, excluding any that are included as "placental remnants."

Significantly different from control; *p<0.05, **p<0.01.

B. DEVELOPMENTAL TOXICITY

The numbers of fetuses (litters) examined in the 0, 40, 200, and 1000 mg/kg/day groups were 155 (17), 138 (18), 132 (16), and 50 (9), respectively. The overall incidence rates for litters containing fetuses with external, visceral, and/or skeletal malformations in the 0, 40, 200, and 1000 mg/kg/day groups were 2/17 (11.8%), 5/18 (27.8%), 7/16 (43.8%), and 0/9, respectively. Selected fetal morphological data are given in Table 5.

1. External examination

One fetus from the 200 mg/kg/day group had multiple external malformations, including craniorachischisis, microphthalmia, open eyelids, cleft palate, and club hand. No external malformations were observed in the 0, 40, or 1000 mg/kg/day groups, and no external developmental variations were observed in any group.

2. Visceral examination

Dilatation of the lateral ventricles was observed in the fetus from the 200 mg/kg/day group that had multiple external malformations. An undescended testis was observed in one fetus from each of the 0, 40, and 200 mg/kg/day groups. There were no visceral malformations observed in the 1000 mg/kg/day group. The only observed visceral variation was thymic remnant in the neck, which occurred in 10 (6), 16 (9), 14 (8), and 5 (2) fetuses (litters) of the 0, 40, 200, and 1000 mg/kg/day groups, respectively.

3. Skeletal examination

The incidence rates of litters containing fetuses with skeletal malformation in the control, low-, mid-, and high-dose groups was 1/17, 4/18, 6/16 ($p<0.05$), and 0/9, respectively. A variety of different malformations were observed with no consistent pattern. No more than two fetuses were affected in any litter of any treatment group. The most commonly observed skeletal variation was lumbar ribs, which were observed in all treated and control groups, though with no statistically significant differences in the fetal or litter incidences. Lumbar rib incidence is within historical control levels, and is a normal background occurrence in the Japanese White rabbit and other strains of rabbits. The skeletal variation 27 presacral vertebrae with 13th ribs was observed at similar fetal and litter incidences in all treated and control groups. Other skeletal variations which occurred at single or low incidences included asymmetry or splitting of the sternebrae, shortening of the 12th ribs, 27 presacral vertebrae without 13th ribs, and 25 presacral vertebrae. The overall fetal incidences of skeletal variations were increased at the 200 and 1000 mg/kg/day treatment levels (41/155, 50/132, and 30/50 fetuses from the 0, 200, and 1000 mg/kg/day groups, respectively; $p<0.05$ and 0.001); however, there were no differences between the overall litter incidences of skeletal variations between the treated and control groups.

TABLE 5: Fetal malformations and variations [Number of litters (fetuses)]				
Observation	**0 mg/kg/day**	**40 mg/kg/day**	**200 mg/kg/day**	**1000 mg/kg/day**
Malformations				
Multiple external malformations [a b]	0/17 (0/155)	0/18 (0/138)	1/16 (1/132)	0/9 (0/50)
Dilatation of the lateral ventricles [b]	0/17 (0/155)	0/18 (0/138)	1/16 (1/132)	0/9 (0/50)
Undescended testis	1/17 (1/155)	1/18 (1/138)	1/16 (1/132)	0/9 (0/50)
Absent skull bones	0/17 (0/155)	0/18 (0/138)	1/16 (1/132)	0/9 (0/50)
Fusion of parietal bones	0/17 (0/155)	1/18 (1/138)	0/16 (0/132)	0/9 (0/50)
Splitting of the parietal bones	0/17 (0/155)	1/18 (1/138)	0/16 (0/132)	0/9 (0/50)
Splitting of the cervical vertebral arches	0/17 (0/155)	1/18 (1/138)	1/16 (1/132)	0/9 (0/50)
Splitting of the thoracic vertebral arches	1/17 (1/155)	0/18 (0/138)	0/16 (0/132)	0/9 (0/50)
Splitting of ossification centers of the thoracic vertebral bodies	0/17 (0/155)	0/18 (0/138)	1/16 (1/132)	0/9 (0/50)
Fusion of the caudal vertebral bodies	0/17 (0/155)	0/18 (0/138)	2/16 (2/132)	0/9 (0/50)
Fusion of the sternebrae	1/17 (1/155)	2/18 (2/138)	2/16 (2/132)	0/9 (0/50)
Bifurcation of the ribs	0/17 (0/155)	0/18 (0/138)	1/16 (1/132)	0/9 (0/50)
Shortening of the femur	0/17 (0/155)	0/18 (0/138)	1/16 (1/132)	0/9 (0/50)
Total with skeletal malformations	1/17 (2/155)	4/18 (4/138)	6/16* (6/132)	0/9 (0/50)
Variations				
Thymic remnant in the neck	6/17 (10/155)	9/18 (16/138)	8/16 (14/132)	2/9 (5/50)
Lumbar ribs	12/17 (32/155)	14/18 (27/138)	14/16 (42/132)	6/9 (23/50)
27 presacral vertebrae with 13th ribs	5/17 (6/155)	5/18 (10/138)	5/16 (6/132)	4/9 (4/50)
27 presacral vertebrae	2/17 (2/155)	0/18 (0/138)	1/16 (1/132)	1/9 (1/50)
Asymmetry of the sternebrae	0/17 (0/155)	0/18 (0/138)	0/16 (0/132)	1/9 (1/50)
Splitting of the sternebrae	0/17 (0/155)	1/18 (1/138)	0/16 (0/132)	1/9 (2/50)
Shortening of the 12th ribs	1/17 (1/155)	0/18 (0/138)	1/16 (1/132)	0/9 (0/50)
25 presacral vertebrae	1/17 (1/155)	0/18 (0/138)	0/16 (0/132)	0/9 (0/50)
Total with skeletal variations	15/17 (41/155)	15/18 (38/138)	14/16 (50/132)	7/9 (30/50)

Data taken from text, pp 15-16 and Tables 8, 9, and 10, pp 30, 31, and 32, respectively, MRID 45014710.

[a]Multiple external malformations included craniorachischisis, microphthalmia, open eyelids, cleft palate, and club hand.

[b]Observed in the same fetus.

[c]These two skeletal malformations were observed in two fetuses from the same litter.

Significantly different from control; * $p<0.05$, ** $p<0.01$, or *** $p<0.001$.

III. DISCUSSION

A. INVESTIGATORS' CONCLUSIONS

The study author concluded that XRD-537 BE resulted in maternal toxicity at 200 and 1000 mg/kg/day as evidenced by maternal mortality, hematuria, body weight losses, and decreased food consumption. Similar findings were not observed in the probe study, in which there was no maternal toxicity at doses up to 1000 mg/kg/day. The study author stated that "although the reason for this inconsistency is not clear at present, it might be caused by a difference in susceptibility between batches of rabbits." The decreased mean numbers of implants and live fetuses at 1000 mg/kg/day were due to increased preimplantation losses. Because treatment began after implantation was believed to have occurred, the lower implantation rate of the 1000 mg/kg/day group was probably not treatment related. The study author stated that the significantly increased litter incidence of total malformations was probably incidental "since 1) statistical significance seemed to be due to a relatively low control value..., 2) type of malformations was not consistent among groups or within a group, and 3) no malformations were found in the highest dose level of 1000 mg/kg." The study author reported that a statistically significant increase in fetal incidence of lumbar ribs was a treatment-related effect based upon a Fisher's exact probability test. The Dow Chemical Company later determined that this was an inappropriate statistical method and performed a renalaysis using the Censored Wilcoxon test (MRID 45413901). Using an appropriate litter-based statistical method that is ideally suited for developmental toxicity incidence data, no evidence of an increased incidence of lumbar ribs was found. The high incidence of lumbar ribs in the concurrent controls (20.6% of fetuses; 70.6% of litters) and among historical controls (8.1-34.6% of fetuses; no litter historical data presented) indicates that lumbar rib is a normal background occurrence in the Japanese White rabbit and in other rabbit strains such as the New Zealand White. The study author concluded that the maternal and developmental toxicity NOAEL was 40 mg/kg/day, and the maternal and developmental toxicity LOAEL was 200 mg/kg/day. The study author also concluded that 1000 mg/kg/day represented an LD_{50} for the maternal rabbits in this study.

B. REVIEWER'S DISCUSSION (in consultation with Stephen Dapson, RAB3, and after HIARC discussion)

1. MATERNAL TOXICITY

It is uncertain whether necropsy findings of "cloudy" or "dark" kidney discoloration and whitish membranous material on the gastric mucosa could have been test article related. There was a higher incidence of hematuria in the 1000 mg/kg/day group during the treatment interval with corresponding decreases in body weight gain and food consumption. It is unlikely that hematuria could have been caused by the test article because it was observed in two animals (one control and one from the 200 mg/kg/day group) prior to the initiation of dosing, and in a second control group animal during the post-dosing interval. If there had been blood in the urine, this would be a serious finding and it should have been identified via urinalysis, but it was

not. It is more likely that hematuria was a misdiagnosis.

According to Harkness and Wagner,[1] normal rabbit urine can be pale yellow to red-orange to brown, and vary from clear to opaque and resemble pus or blood. The color of rabbit urine, caused by porphyrin and bilirubin derivatives, is intensified during dehydration or on certain pigmented or high calcium diets. Bloody urine is very rare in rabbits and rodents. If the urine is indeed bloody, then cystitis (inflammation of the urinary bladder), urolithiasis (calculi), and leptospirosis should be considered. Most cases of "bloody" urine turn out to be a porphyrin-pigmented basic urine or a sanguineous vaginal discharge from a tumor or abortion. Considering the pattern of occurrence, what was reported as hematuria was probably concentrated, blood-colored urine which can be attributed to dehydration, rather than to disease or the test article.

The maternal LOAEL is 200 mg/kg/day based on maternal death. The maternal NOAEL is 40 mg/kg/day.

2. DEVELOPMENTAL TOXICITY

a. Deaths/resorptions

There were no increases in mean numbers of fetal deaths or resorptions per litter.

b. Altered growth

No evidence of altered growth of the fetuses was seen in this study.

c. Developmental variations

The most commonly observed skeletal variation was lumbar ribs, which were observed in all treated and control groups, though with no statistically significant differences in the fetal or litter incidences. Lumbar rib incidence is close to historical control levels, and is a normal background occurrence in the Japanese White rabbit and other strains of rabbits.

d. Malformations

The increased litter incidences of skeletal malformations and total malformations at the 200 mg/kg/day dose level were most likely due to maternal stress rather than test article toxicity.

The developmental NOAEL is ≥1000 mg/kg/day (limit dose).

---

[1]John E. Harkness and Joseph E. Wagner. **The Biology and Medicine of Rabbits and Rodents.** 2nd Edition. Lea & Febiger, Philadelphia. 1983.

C. STUDY DEFICIENCIES

The study report did not describe dose formulation preparation or storage conditions, and there was no mention made of stirring the dosing solutions during dosing to maintain homogeneity. Nevertheless, non-homogeneity was not a problem in any other toxicity studies, and the dosing measurements suggest that the formulations were homogeneous. Ideally, more rabbits should have been used. Since this study was performed prior to implementation of the OPPTS Harmonized Test Guidelines, only 12 rabbits per group are required. Randomization procedures and sacrifice order were not provided. The study report did not include a copy of the study protocol and any amendments.

D. CORE CLASSIFICATION

This study is classified as **Acceptable/Guideline** and does satisfy the requirements for a developmental toxicity study [OPPTS: 870.3700 (§83-3)] in rabbits.

DATA EVALUATION RECORD

XRD-537 BE (CYHALOFOP BUTYL)
STUDY TYPE: MULTIGENERATION FEEDING - RAT [OPPTS 870.3800 (§83-4)]
MRID 45000419

Prepared for

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Office of Pesticide Programs
U.S. Environmental Protection Agency
1921 Jefferson Davis Highway
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Prepared by

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Disclaimer

This review may have been altered subsequent to the contractor's signatures above.

CYHALOFOP BUTYL

Reproduction Study [OPPTS 870.3800 (§83-4)]

EPA Reviewer: John E. Whalan
Registration Action Branch 2 (7509C)
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Registration Action Branch 2 (7509C)
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Date 6-5-02

Date 6/5/02

DATA EVALUATION RECORD

STUDY TYPE: Multigeneration Reproduction Study - Rat; [OPPTS 870.3800 (§83-4)]

DP BARCODE: D268553
P.C. CODE: 082583

SUBMISSION CODE: none
TOX. CHEM. NO.: none

TEST MATERIAL (PURITY): XRD-537 BE (Cyhalofop butyl) (97.1 a.i.)

SYNONYMS: R-(+)-n-butyl-2-(4-(2-fluoro-4-cyanophenoxyl)phenoxy)propanoate

CITATION: Aoyama, H., et al. 1994. Two-generation reproduction study in rats with XRD-537 BE. Mitsukaido Laboratories, The Institute of Environmental Toxicology, Ibaraki 303, Japan. Laboratory study ID, GHF-P-1388, May 23, 1994, MRID 45000419. Unpublished.

SPONSOR: DOW AgroSciences, LLC

EXECUTIVE SUMMARY:

In a two-generation reproduction study (MRID 45000419), XRD-537 BE [(97.1% a.i., lot no. DECO-26-42T (AGR 295713)] was administered to groups of 24 male and 24 female F_0 and F_1 rats at dietary levels of 0, 10, 100, or 1000 ppm. The dietary levels of XRD-537 BE for F_0 males corresponded to 0, 0.495-1.049, 4.88-10.68, or 50.0-102.9 mg/kg/day, respectively, and 0, 0.499-1.361, 4.85-13.75, or 51.1-138.7 mg/kg/day, respectively, for F_1 males for the entire study duration. The dietary levels for F_0 females corresponded to 0, 0.695-1.113, 6.75-11.13, or 69.2-113.1 mg/kg/day, respectively, and 0, 0.750-1.430, 7.42-13.96, or 74.8-147.7 mg/kg/day, respectively, for F_1 females for the premating period. Each group received treated or control diet for 10 weeks before mating and during mating, gestation, and lactation of one litter per generation. The F_1 pups selected to parent the F_2 generation were weaned onto the same dietary level as their parents.

No treatment-related deaths, clinical signs, body weight changes, or food consumption differences were observed for parental male or female rats in either generation administered any dose of the test material. No effects were observed for F_0 or F_1 females during gestation or lactation. Postmortem examination of parental rats showed that absolute and relative liver weights were significantly increased by 22-28% and absolute and relative kidney weights were significantly increased by 10-13% in high-dose group F_0 and F_1 males. Absolute and relative liver weights were increased by 11-17% in high-dose group female rats in both generations. Gross examination of adult rats showed that the livers in F_1 high-dose group males and females

were enlarged and/or cloudy in color in 14-21 (58-88%) rats per group compared with none in any control group. Microscopic examination showed that all high-dose group male and female rats, but no controls, in both generations had hepatocellular hypertrophy that was considered an adaptive response. All high-dose group male rats, but none of the controls, in both generations had slight renal tubular swelling. The absolute and relative kidney weights were increased in mid-dose F_1 male rats, but no corresponding gross or microscopic lesions were observed.

Dietary administration of the test material had no effects on reproductive performance; mating index for both sexes, fertility index, gestation index, and duration of gestation were comparable in treated and control groups. A slight, but significant increase in the implants/dam in high-dose group F_0 female rats was associated with a slight, but significant increase in the number of pups/litter. Consequently, mean pup weight on lactation day 0 was slightly, but significantly decreased for the F_1 high-dose group. Other than a slight decrease in F_1 pup weight on lactation day 21, no other statistically significant difference in pup weight were observed for either the F_1 or F_2 generation at any time during lactation. Survival indices were similar in treated and control pups throughout lactation.

Systemic NOAEL (males) = 100 ppm (4.85-13.75 mg/kg/day)
Systemic LOAEL (males) = 1000 ppm (50.0-138.7 mg/kg/day, HDT) based on kidney lesions (slight tubular cell swelling) in F_0 and F_1 male rats.
Systemic NOAEL (females) ≥1000 ppm (69.2-147.7 mg/kg/day, HDT)
Reproductive NOAEL ≥1000 ppm (50.1-138.7 mg/kg/day for males; 69.2-147.7 mg/kg/day for females, HDT)
Offspring NOAEL ≥1000 ppm (50-147.7 mg/kg/day, HDT)

The animals were adequately dosed to assess reproductive toxicity as evidenced by hepatocellular hypertrophy and the induction of kidney lesions in parental rats of both generations.

The reproductive study in the rats is classified **Acceptable/Guideline** and does satisfy the guideline requirement for a two-generation reproductive study [OPPTS 870.3800,(§83-4)] in the rat.

COMPLIANCE: Signed and dated GLP, Quality Assurance, Data Confidentiality, and Flagging statements were provided.

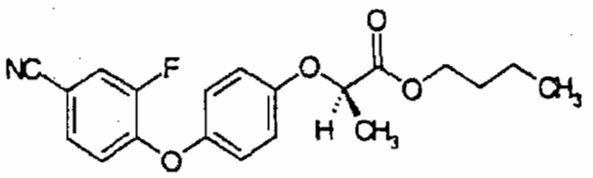
I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: XRD-537 BE

Description: off-white powder
Lot/Batch No.: DECO-26-42T (AGR 295713)
Purity: 97.1% a.i.
CAS No.: 122008-85-9

Structure:



2. Vehicle: The test material was administered in pulverized feed NMF (Oriental Yeast Co. Ltd.)

3. Test animals

Species: Rat, specific pathogen free
Strain: Crj:CD (SD)
Age at start of dosing: F_0, ~5 weeks
Weight at start of dosing: F_0: males: 136-152 g; females: 118 - 134 g
Source: Charles River Japan, Inc.
Housing: During acclimation: five per sex in suspended wire-mesh stainless-steel cages
Premating: 3 of one sex in suspended wire-mesh stainless-steel cages
Mating (males): individually housed in aluminum cages with wire-mesh floors and fronts
Gestation/lactation: mated females housed individually in aluminum boxes with nesting material
Diet: pulverized NMF feed (Oriental Yeast Co. Ltd.) *ad libitum*
Water: well water subjected to precipitation and sedimentation procedures and sterilized with hypochlorous acid and ultraviolet light, *ad libitum*
Environmental conditions:
Temperature: 22°C ± 2°C
Humidity: 55% ± 10%
Air changes:19/hr
Photoperiod: 12 hours dark/12 hours light
Acclimation period (F_0): 7 days

B. PROCEDURES AND STUDY DESIGN

1. In life dates

Start: August 4, 1992; end: April 12, 1993

2. Mating procedure

One female in the proestrous stage and one male of the same dose group were cohabited in the cage of the male overnight and examined for evidence of mating (copulatory plug or sperm in vaginal smear) the following morning. If no evidence of mating was found, the females were checked daily for stage of the estrous cycle;

when the female was in proestrous, she was again cohabited with the same male rat and checked for evidence of mating. This procedure was repeated until evidence of mating was found or for 3 weeks. After mating was confirmed, the females were placed in breeding boxes and the day evidence was confirmed was designated day 0 of gestation. F_1 rats were mated as described for F_0; sibling matings were avoided.

3. Study schedule

Test or control diet was administered continuously to each group of F_0 females for 10 weeks before mating and during mating, gestation, and lactation. F_0 males were administered the same diets continuously for 10 weeks before mating and throughout the study until sacrifice after weaning their offspring. One or two 21- to 24-day old F_1 weanlings from each litter born within a 4-day period were selected to produce the F_2 generation. A total of 24 male and 24 females were selected from each treatment and control group. F_1 females selected to parent the next generation were administered the same diet as their parents for 10 weeks starting at weaning and continuing throughout mating, gestation and lactation. F_1 males selected to sire the next generation were administered the same diet as their parents for 10 weeks starting at weaning and continuing until sacrifice after weaning of their offspring.

4. Animal assignment

F_0 rats were assigned randomly to each test group as presented in Table 1 based on body weight so that group means and standard deviations were equal.

TABLE 1. Animal assignment

Test group	Concentration in diet[a] (ppm)	Animals/group			
		F_0 Males	F_0 Females	F_1 Males	F_1 Females
Control	0	24	24	24	24
Low (LDT)	10	24	24	24	24
Mid (MDT)	100	24	24	24	24
High (HDT)	1000	24	24	24	24

Data were taken from pages 7 and 9, MRID 4500419.

[a]Diets were administered from the beginning of the study (F_0) or from weaning (F_1) until sacrifice after weaning of offspring.

5. Dose selection rationale

Dose selection was based on a one-generation preliminary study in which groups of eight male and eight female rats were administered diets containing 0, 100, 300, 1000, or 3000 ppm of XRD-537 BE starting 3 week before mating and continuing until weaning of the F_1 offspring. Liver weights were increased and were observed to be enlarged and darkened upon gross examination at 3000 ppm in both sexes. Liver weights were increased at all doses, even at the lowest dose in males. In addition, the

relative kidney weight was significantly increased in males at 3000 ppm. No treatment-related effects were observed in the offspring.

6. Dosage preparation and analysis

Formulations were prepared monthly. Specified amounts of test substance were dissolved in 200 g acetone (>99% purity), mixed with a small amount of feed in a mortar, and mixed (SS-501 mixer) with the remaining portion of feed to obtain the specified dietary concentration. The same amount of acetone was added to the control diet. Acetone was allowed to evaporate for 30 minutes. The prepared diets were stored in a cold room until used. Fresh diet was offered weekly. At study initiation, homogeneity and stability were determined on the 10-ppm diet. Homogeneity was evaluated on samples taken from the top, middle, and bottom of the mixer, and stability of the test substance was evaluated on samples stored at 4°C in the dark for 28 days with and without an additional 21 days at room temperature. During the study, samples of treated food were analyzed for concentration at monthly intervals.

Results

Homogeneity Analysis: The range of concentration in samples taken from the top, middle, and bottom of the mixer was 8.9 to 9.6 ppm; the coefficient of variation was 2.2%.

Stability Analysis: The mean concentration in the samples stored for 28 days at 4°C was 2% less than the concentration on day 0 and 4% less after storage at 4°C for 28 days followed by room temperature for an additional 21 days.

Concentration Analysis: The mean concentration of test material at all three dietary concentrations varied by no more than -9% to +6% of the target concentrations.

The analytical data indicated that the mixing procedure was adequate and that the variance between nominal and actual dosage to the study animals was acceptable.

C. OBSERVATIONS

1. Parental animals

All animals were examined daily for clinical signs of toxicity and mortality; a detailed examination was conducted weekly. Animals found dead or killed moribund were subjected to a gross examination. Estrous cycles were evaluated from vaginal smears taken for 1 or more weeks. Female rats were weighed at study initiation, weekly during premating, on gestation days 0, 7, 14, and 20, lactation days 0, 7, 14, and 21, and at necropsy. Male rats were weighed at study initiation, weekly during premating, and biweekly during the "breeding" period. Food consumption for each cage was measured weekly during the premating period for all groups and at 2-week intervals during the breeding (postmating) period (except for the mating period) for males.

During gestation, food consumption for females was measured for the following intervals: days 0-7, 7-14, and 14-20; and during lactation: days 0-7, 7-14, and 14-21. Food consumption was calculated as g food/rat/day. Food efficiency was not calculated. Compound intake was calculated from food consumption and body weight data.

2. Litter observations

The following litter observations (X) were made (Table 2). On day 4 postpartum, litters were culled to four males and four females (if possible). The excess pups were euthanized, necropsied, and discarded.

TABLE 2. F_1/F_2 Litter observations

Observation	Time of observation (lactation day)					
	Day 0	Day 4[a]	Day 4[b]	Day 7	Day 14	Day 21
Clinical signs	Daily					
Number of live pups	X	X				X
Pup weight	X	X		X	X	X
External alterations	X	X		X	X	X
Number of dead pups	X					
Sex of each pup (M/F)	X					

Data extracted from pages 17-19, MRID 4500419.
[a]Before standardization (culling)
[b]After standardization (culling)

3. Postmortem observations

a. Parental animals

All surviving F_0 and F_1 parental male and female rats were sacrificed and necropsied after weaning of their offspring. A pair that did not produce offspring were sacrificed and necropsied at the same time as other parental animals. The following tissues (X) were collected for microscopic examination. The head, which included the nasal cavity, paranasal sinuses, buccal mucosa and ears also were prepared for microscopic examination. Testes, epididymides, seminal vesicles, and prostate from male rats and ovaries, uteri, and vagina from female rats in the control and high-dose groups were examined microscopically. Tissues from animals that died before mating were not examined. Reproductive organs from animals in the low- and mid-dose groups were examined if they failed to mate, produce offspring, or had an abnormal delivery (females). The liver and kidneys of animals in the control, mid-, and high-dose groups were examined microscopically. The (XX) organs from 10 males and 10 females of each genera-

tion were weighed. The seminal vesicle, coagulating glands, and fluid were weighed together.

X	DIGESTIVE SYSTEM	X	CARDIOVASC./HEMAT.	X	NEUROLOGIC
X	Tongue	X	Aorta	XX	Brain
X	Salivary glands	X	Heart		Periph. nerve
X	Esophagus		Bone marrow		Spinal cord (3 levels)
X	Stomach		Lymph nodes	XX	Pituitary
X	Duodenum	X	Spleen	X	Eyes (optic n.)
X	Jejunum	X	Thymus		
X	Ileum				GLANDULAR
X	Cecum		UROGENITAL	XX	Adrenal gland*+
X	Colon	XX	Kidneys		Lacrimal gland
X	Rectum	X	Urinary bladder		Harderian gland
XX	Liver	XX	Testes*	X	Mammary gland
X	Pancreas	XX	Epididymides*	X	Parathyroids
		XX	Prostate*	X	Thyroids
	RESPIRATORY	XX	Seminal vesicle*		
X	Trachea	XX	Coagulating gland		OTHER
X	Lung	XX	Ovaries*		Bone
X	Nose	XX	Uterus*		Skeletal muscle
X	Pharynx	X	Oviduct		Skin
X	Larynx	X	Cervix	X	Ears
		X	Vagina	X	All gross lesions and masses*

Data taken from pages 15-16, MRID 4500419.
*Required for reproductive toxicity studies based on Subdivision F Guidelines.

a. Offspring

Culled offspring were euthanized and necropsied, and F_1 weanlings not selected to parent the next generation and all F_2 weanlings were euthanized and necropsied when 21-27 days old. Gross findings were recorded and the tissues and organs were discarded after gross examination.

D. DATA ANALYSIS

1. Statistical analyses

Parental body weights, food consumption, and organ weights, mean number of implants, number of pups delivered, and pup body weight were analyzed by Student's or Aspin-Welch's t-test. Incidence data (clinical signs, gross and histopathologic lesions, reproductive indices, and sex ratios) were analyzed by Fisher's exact probability test. Viability indices and duration of gestation were analyzed by the Mann-Whitney's U-test. Statistical significance was indicated by $p \leq 0.05$.

2. Indices

Reproductive indices

The following reproductive indices were calculated from breeding and parturition records of animals in the study. Copulation was indicated by sperm in the vaginal smears and/or the presence of a vaginal plug. A pregnancy was evidenced by parturition or implantation sites in the uterus.

$$\text{Male (female) mating index (\%)} = \frac{\text{No. males (females) that copulated}}{\text{No. males (females) mated}} \times 100$$

$$\text{Fertility index (\%)} = \frac{\text{No. pregnant}}{\text{No. that copulated}} \times 100$$

$$\text{Gestation index} = \frac{\text{No. of normal parturitions}}{\text{No. pregnant}} \times 100$$

Offspring viability indices: The following viability indices were calculated from lactation records of litters in the study:

$$\text{Live birth index (\%)} = \frac{\text{No. pups alive on lactation day 0}}{\text{No. pups delivered}} \times 100$$

$$\text{Viability index (\%)} = \frac{\text{No. pups alive on day 4 (precull)}}{\text{No. alive on day 0}} \times 100$$

$$\text{Lactation index (\%)} = \frac{\text{No. pups alive on day 21}}{\text{No. pups alive on day 4 (postcull)}} \times 100$$

3. Historical control data

Historical control data were not provided in this report.

II. RESULTS

A. PARENTAL ANIMALS

1. Mortality and clinical signs

All female rats in both generations survived to termination except for one female in the mid-dose group. This animal was killed *in extremis* on day 20 of gestation after having a red vaginal discharge, anemia, piloerection and decreased motor activity. A total of six male rats died or were killed *in extremis* (two high-dose F_0, two high-dose F_1, and two F_1 controls); five died due to complications from a broken nasal bone (may indicate rough handling of the animals) and one died due to an unknown cause.

No treatment-related clinical signs of toxicity were observed in any group of F_0 or F_1 rats of either sex. Six F_0 male rats in the 10-ppm group had hair loss during the breeding (postmating) period.

2. Body weight and food consumption

Selected mean body weight, body weight gain, and food consumption data are presented in Tables 3a (F_0 generation) and 3b (F_1 generation). Mean absolute body weights and weight gain were similar between all treated groups and the corresponding controls for F_0 and F_1 generation rats. Except for a few statistically significant values, food consumption was similar between all treated groups in both generations and the corresponding control groups. High dose F_1 male rats consumed 5% ($p \leq 0.05$), 7% ($p \leq 0.05$), and 5% ($p \leq 0.05$) more food than the controls at weeks 4, 5, and 6, respectively, and low-dose F_1 females consumed 8% ($p \leq 0.05$) less food than the controls during week 9. Food efficiency was not calculated by the study authors.

TABLE 3a. Body weight, weight gain, and food consumption during the premating and postmating periods in F_0 rats fed XRD-537 BE

Observations/study week	Dietary concentration (ppm)			
	0	10	100	1000
F_0 Generation Males - Premating/Postmating				
Mean body weight (g)				
Week 0	144	144	144	144
Week 5	402	398	405	402
Week 10	508	502	510	503
Week 18	579	570	586	576
Mean weight gain (g)[a]				
Weeks 0-10	364	358	366	359
Weeks 0-18	435	426	442	432
Mean food consumption (g/animal/day)				
Week 1	21.6	21.5	22.1	21.6
Week 10	27.5	27.8	27.6	27.9
Week 16	28.0	27.6	28.0	28.1
F_0 Generation Females - Premating				
Mean body weight (g)				
Week 0	126	126	126	126
Week 5	243	244	246	246
Week 10	292	292	295	299
Mean weight gain (g)[a]				
Weeks 0-10	166	166	169	173
Mean food consumption (g/animal/day)				
Week 1	17.6	17.8	17.7	18.1
Week 10	20.7	20.3	19.9	20.7

Data extracted from Tables 4-7 (pp. 33-36), MRID 4500419.
[a]Calculated by the reviewer.

TABLE 3b. Body weight, weight gain, and food consumption during the premating and postmating periods in F_1 rats fed XRD-537 BE

Observations/study week	Dietary concentration (ppm)			
	0	10	100	1000
F_1 Generation Males - Premating/Postmating				
Mean body weight (g)				
Week 0	74	74	74	74
Week 5	359	360	372	373
Week 10	515	509	523	521
Week 18	604	607	620	631
Mean weight gain (g)[a]				
• Weeks 0-10	441	435	449	447
Weeks 0-18	530	533	546	557
Mean food consumption (g/animal/day)				
Week 1	16.1	16.2	16.5	16.5
Week 10	30.3	29.5	30.2	30.9
Week 16	29.2	29.2	28.9	31.0
F_1 Generation Females - Premating				
Mean body weight (g)				
Week 0	69	69	69	69
Week 5	233	231	236	238
Week 10	307	300	310	313
Mean weight gain (g)[a]				
Weeks 0-10	238	231	241	244
Mean food consumption (g/animal/day)				
Weeks 1	15.2	15.3	14.8	15.8
Week 10	23.1	22.5	23.0	23.4

Data extracted from Tables 4-7 (pp. 33-36), MRID 4500419.
[a]Calculated by the reviewer.

Group mean body weights and food consumption values for pregnant or lactating dams in both generation were similar to those of control rats with a few exceptions. High-dose F_0 females weighed 6% ($p \leq 0.05$) more than controls on day 20 of gestation, and high-dose F_1 females weighed 4% ($p \leq 0.05$) more than controls on lactation day 21 and consumed 18% ($p \leq 0.01$) more food during gestation days 0-7.

3. Test substance intake

The range of values for compound consumption by male and female rats are presented in Table 4. The study authors did not calculate average doses. The high values for the lactation period are due in part to consumption of food by pups during the latter stage of lactation.

TABLE 4. Test Substance Intake (mean mg/kg body weight/day)						
Treatment Period	Male[a]			Female[b]		
	10 ppm	100 ppm	1000 ppm	10 ppm	100 ppm	1000 ppm
F_0 Generation						
Premating	0.495–1.049	4.88–10.68	50.1–102.9	0.695–1.113	6.75–11.13	69.2–113.1
Gestation	–	–	–	0.640–0.741	6.13–7.09	62.3–70.8
Lactation	–	–	–	1.275–2.383	12.39–23.14	125.2–229.1
F_1 Generation						
Premating	0.499–1.361	4.85–13.75	51.1–138.7	0.750–1.430	7.42–13.96	74.8–147.7
Gestation	–	–	–	0.641–0.739	6.71–7.58	67.2–75.7
Lactation	–	–	–	1.196–2.380	12.20–23.58	119.3–229.0

Data taken from Table 8 (pp. 37-39), MRID 4500419.
[a]Range for premating and postmating periods.
[b]Range for premating period.

4. Reproductive function

a. Estrous cycle length and periodicity

No data were collected for determining proestrous stage for mating purposes.

b. Sperm measures

Sperm were not evaluated in this study.

c. Sexual maturation (F_1)

The time of sexual maturation was not determined in this study.

5. Reproductive performance

Data for reproductive performance of F_0 and F_1 generation rats are summarized in Table 5. No treatment-related effects were observed on any parameter of reproductive performance. High-dose F_0 female rats had a significantly larger number of implants than the controls.

TABLE 5. Reproductive performance for F_0 and F_1 generation rats fed XRD-537 BE

Observation	Dietary concentration (ppm)			
	0	10	100	1000
F_0 Generation				
Males				
No. at start of study	24	24	24	24
No. paired with females	24	24	24	24
Intercurrent deaths	0	0	0	2
Females				
No. at start of study	24	24	24	24
No. paired with males	24	24	24	24
Intercurrent deaths	0	0	1	0
Median gestation interval (days)	22.3	22.2	22.2	22.1
No. implants/dam	15.0	15.3	15.9	16.3*
INDICES (%)				
Mating (male)	95.8	100	100	100
Mating (female)	95.8	100	100	100
Fertility	95.7	100	100	100
Gestation	100	100	95.8	100
F_1 Generation				
Males				
No. at start of study	24	24	24	24
No. paired with females	22	23	24	23
Intercurrent deaths	2	0	0	2
Females				
No. at start of study	24	24	24	24
No. paired with males	24	24	24	24
Intercurrent deaths	0	0	0	0
Median gestation interval (days)	22.2	22.2	22.3	22.3
No. implants/dam	15.6	14.7	15.0	14.8
INDICES (%)				
Mating (male)	100	95.7	100	100
Mating (female)	100	95.8	100	100
Fertility	100	100	87.5	87.5
Gestation	100	100	100	100

Data taken from Tables 1 (page 30) and 9 (page 40), MRID 4500419.
*$p \leq 0.05$, statistically significant, treated group compared with controls.

5. Parental postmortem results

a. Organ weights

Selected organ weights are summarized in Table 6. In high-dose group males in both generations, absolute and relative liver weights were increased by 22-28% ($p \leq 01$) and absolute and relative kidney weights were increased by 10-13% ($p \leq 0.01$ or ≤ 0.05). In addition, absolute and relative kidney weights were increased by 11% and 6%, respectively, in the mid-dose group F_1 male rats. Absolute and relative liver weights were increased in the high-dose group F_0 females by 17% and 13% ($p \leq 0.05$), respectively, and in the high-dose group F_1 female rats by 11% (N.S.) and 12% ($p \leq 0.05$), respectively. The high-dose group F_1 female rats also had increased absolute brain weight (3%, $p \leq 0.05$), decreased absolute and relative pituitary weights (14% and 12%, respectively, $p \leq 0.05$), and increased absolute and relative ovarian weight (20% and 22%, respectively, $p \leq 0.01$ and ≤ 0.05) compared with the control weights. Statistically significant changes in the weight of other organs were observed but the lack of dose-response relationships suggested that the changes were not treatment related.

TABLE 6. Selected absolute and relative mean organ weights for F_0 and F_1 male and female rats fed XRD-537 BE

Organ	Dietary Concentration (ppm)			
	0	10	100	1000
F_0 Generation				
Males				
Liver (g)	21.041[a] 3.67	19.561 3.50	22.109 3.81	26.922** (128)[b] 4.58** (125)
Kidney (g)	1.885 0.331	1.803 0.324	1.961 0.339	2.131** (113) 0.365* (110)
Females				
Brain (g)	2.002 0.622	2.046 0.634	2.018 0.635	2.048 0.622
Pituitary (mg)	16.4 0.00511	18.3 0.00566	18.3 0.00575	17.4 0.00522
Liver (g)	15.971 4.96	17.441 5.39	16.510 5.13	18.691* (117) 5.58* (113)
Ovary (mg)	29.0 0.0182	59.3 0.0183	64.6 0.0203	64.8 0.0196
F_1 Generation				
Males				
Liver (g)	23.334 3.84	22.944 3.78	25.481 3.96	28.644** (123) 4.68** (122)
Kidney (g)	1.990 0.327	2.005 0.332	2.212* (111) 0.345* (106)	2.197* (110) 0.360** (110)
Females				
Brain (g)	2.026 0.596	2.049 0.620	2.006 0.601	2.096* (103) 0.623 (105)
Pituitary (mg)	19.0 0.00554	19.3 0.00581	18.7 0.00560	16.3* (86) 0.00485* (88)
Liver (g)	17.325 5.06	17.221 5.15	17.162 5.11	19.255 (111) 5.67* (112)
Ovary (mg)	60.4 0.0177	63.0 0.0190	61.8 0.0185	72.7** (120) 0.0216* (122)

Data taken from Tables 12, 13, 14, and 15 (pp. 44-47), MRID 4500419.

[a]Absolute weight (in g or mg) in top row and relative weight (as % body weight) in bottom row.

[b]Numbers in parentheses are percent of control, calculated by the reviewer.

*$p \leq 0.05$, **$p \leq 0.01$, statistically significant, treated group compared with controls.

b. Pathology

1. Macroscopic examination

No treatment-related gross lesions were observed in F_0 rats of either sex. In the F_1 generation, the liver was enlarged in 14/24 (58%) males and 21/24 (88%) females and the liver was cloudy in color in 19/24 (79%) males and 16/24 (67%) females at the high-dose level. These lesions were not observed in the control, low-dose, or mid-dose groups. The remaining gross findings occurred with similar incidences in treated and control groups.

2. Microscopic examination

All male and female F_0 and F_1 rats in the high-dose group had diffuse hepatocellular swelling in the liver that was moderate in males and slight in all females. This finding did not occur in the control or mid-dose groups. In addition, one high-dose F_1 male rat also had moderate focal hepatocellular necrosis. Tissues from low-dose group animals were not examined microscopically. All high-dose group F_0 and F_1 male rats had slight renal tubular cell swelling; this finding did not occur in control or mid-dose group rats.

B. OFFSPRING

1. Viability and clinical signs

The only clinical signs that occurred in a significantly greater number of treated pups than in controls were swelling of the umbilical region of F_1 pups and loss of F_1 pups due to cannibalism in the high-dose group. Swelling of the umbilical region occurred in 8/192 ($p \leq 0.01$) 5- to 7-days old pups compared with none of the 175 control pups. The incidence showed a progressive decrease as the pups aged, by lactation days 8-14 only 4/192 had swelling of the umbilical region and by lactation days15-21 only 2/192 pups exhibited this sign. A total of 15 F_1 pups ($p \leq 0.05$) in the high-dose group were lost due to cannibalism between day 1 and 4 of lactation compared with only 4 controls

Mean litter size and F_1 and F_2 pup survival indices during lactation are summarized in Table 7. No treatment-related effects occurred on the mean litter size at birth, live birth, viability, or lactation indices, or the sex ratios of either F_1 or F_2 pups. Significantly fewer low-dose group F_2 pups were found dead on day 0 compared with the number of control pups.

TABLE 7. Mean litter size and viability during lactation of F_1 and F_2 pups				
Observation	**Dietary Concentration (ppm)**			
	0	**10**	**100**	**1000**
F_1 Generation Litters				
No. litters[a]	22	24	23	24
Total No. of pups day 0	293	334	326	369
Mean No. pups/litter, day 0[b]	13.3	13.9	14.2	15.4**
No. found dead, day 0	10	16	13	16
Survival indices				
Live birth index	96.8	94.9	95.5	96.0
Viability index (PND 1-4)	98.5	99.2	96.6	95.7
Lactation index (PND 4-21)	97.2	99.4	100.0	100.0
Sex ratio at birth (males/females)	0.471	0.530	0.494	0.488
F_2 Generation Litters				
No. litters[a]	24	23	21	21
Total No. of pups day 0	338	300	280	287
Mean No. pups/litter, day 0[b]	14.1	13.0	13.3	13.7
No. found dead, day 0	20	7*	12	13
Survival indices				
Live birth index	94.5	97.6	96.3	95.8
Viability index (PND 1-4)	98.4	98.5	98.5	98.4
Lactation index (PND 4-21)	98.4	100.0	100.0	100.0
Sex ratio at birth (males/females)	0.464	0.530	0.443	0.502

Data extracted from Tables 9 (page 40) and 18 (page 51), MRID 4500419.
[a]Same as number of normal parturitions listed in Table 9 (page 40).
[b]Same as the mean number of pups delivered /litter listed in Table 9 (page 40).
PND = postnatal day
*p≤0.05, **p≤0.01, statistically significant, treated groups compared with the control.

2. Body weight

Mean pup body weight data are presented in Table 8. Pup weights were calculated as the mean of the average weights of the pups in each litter. Mean body weights were slightly, but significantly reduced by 7% (p≤0.01) in high-dose F_1 male pups and by 6% (p≤0.01) in high-dose F_1 female pups on day 0 . In addition, high-dose F_1 female pups weighed 5% (p≤0.05) less than controls on day 21 of lactation. No other statistical differences were observed at any time during lactation for F_1 or F_2 pups in any dose group.

TABLE 8. Mean body weight and body weight gain for F_1 and F_2 generation pups

Day of lactation	Dietary Concentration (ppm)							
	0	10	100	1000	0	10	100	1000
F_1 Generation Pups								
Mean pup weight (g)	Male pups				Female pups			
Day 0	7.3	7.0	7.0	6.8**	6.8	6.7	6.6	6.4**
Day 4	12.1	12.2	11.7	11.5	11.5	11.9	11.3	11.0
Day 7	19.3	19.8	19.3	18.6	18.7	19.2	18.6	17.7
Day 14	38.3	38.7	37.7	36.9	37.0	37.6	36.6	35.3
Day 21	62.8	63.9	60.4	60.0	59.5	60.7	57.9	56.3*
Pup weight gain (g)								
Day 1-4	4.8	5.2	4.7	4.7	4.7	5.2	4.7	4.6
Day 4-7	7.2	7.6	7.6	7.1	7.2	7.3	7.3	6.7
Day 7-14	19.0	18.9	18.4	18.3	18.3	18.4	18.0	17.6
Day 14-21	24.5	25.2	22.7	23.1	22.5	23.1	21.3	21.0
Day 1-21	55.5	56.9	53.4	53.2	52.7	54.0	51.3	49.9
F_2 Generation Pups								
Mean pup weight (g)	Male pups				Female pups			
Day 0	7.3	7.2	7.4	7.2	6.8	6.8	7.0	6.7
Day 4	12.3	12.2	12.6	12.3	11.9	11.7	12.1	11.8
Day 7	20.0	19.8	20.7	19.8	19.2	18.9	19.7	19.0
Day 14	40.8	40.1	41.3	40.1	39.4	38.2	39.1	38.6
Day 21	68.9	67.6	69.8	67.2	65.5	63.7	65.2	64.3
Pup weight gain (g)								
Day 1-4	5.0	5.0	5.2	5.1	5.1	4.9	5.1	5.1
Day 4-7	7.7	7.6	8.1	7.5	7.3	7.2	7.6	7.2
Day 7-14	20.8	20.3	20.6	20.3	20.2	19.3	19.4	19.6
Day 14-21	28.1	27.5	28.5	27.1	26.1	25.5	26.1	25.7
Day 1-21	61.6	60.4	62.4	60.0	58.7	56.9	58.2	57.6

Data taken from Table 20, page 53, MRID 4500419.
*p<0.05, **p<0.01, statistically significant compared with controls.

3. Offspring postmortem results:

   a. Organ weights: Organs in pups were not weighed in this study.

   b Pathology

1. Macroscopic examination:

The only finding that showed a significantly increased incidence was thymic remnant in the neck of 9 ($p \leq 0.05$) mid-dose F_2 pups killed on lactation day 4 compared with only 2 controls. In addition, 14 ($p < 0.05$) low-dose F_2 pups found dead between lactation days 5 and 21 had thymic remnants in the neck compared with 6 controls. The lack of a dose-response relationship suggest that this finding is not related to treatment with the test material.

2. Microscopic examination:

Tissues and organs from pups were not examined microscopically.

III. DISCUSSION

A. INVESTIGATORS' CONCLUSIONS

The study authors concluded that feeding of XRD-537 BE for two generations caused no parental effects at 10 ppm. Minimal toxic effects were observed at 100 ppm in F_1 parental male rats manifested as elevated absolute and relative kidney weights. Definite toxic effects were observed in the kidney and liver of both sexes and generations at 1000 ppm. Kidney weights were significantly increased and renal tubular cell swelling was observed microscopically. Absolute and/or relative liver weights were significantly increased in both sexes and generations. The liver appeared enlarged and cloudy in color in the F_1 generation upon gross examination. Microscopically, hepatocellular swelling was observed in all rats in both generations. No treatment-related effects were observed on reproductive performance of parental animals or on the pups during lactation. The study authors concluded that the no-observed-effect level was 10 ppm for parental animals and 1000 ppm for the offspring.

B. REVIEWER'S DISCUSSION

Administration of XRD-537 BE at a dietary concentration of 10, 100, or 1000 ppm caused no treatment-related mortality, clinical signs, changes in absolute body weight gain, or food consumption at any dose. High-dose group F_1 males consumed slightly, but significantly more food during weeks 4, 5, and 6 of the premating period. This transient increase is not considered treatment related nor toxicologically significant, because no effect was observed on body weight gain. Postmortem examination of parental animals showed that the kidney and liver were affected by the test material. The absolute and relative liver weights were increased in high-dose group rats in both sexes and both generations, and the kidney weights were increased in the high-dose group male rats in both generations and mid-dose group male rats in the F_1 generation. The relative organ weights were increased because terminal body weights of treated animals were comparable with that of controls. The majority of F_1 rats of both sexes had enlarged livers that were cloudy in appearance, and all treated rats in the high-dose group had microscopic

lesions in the liver described as hepatocyte swelling (hypertrophy) that was moderate in all males and slight in all females. Although one F_1 high-dose male rat had moderate focal hepatocellular necrosis, data from other toxicity studies for this chemical suggest that the hepatocellular hypertrophy was an adaptive response. All F_0 and F_1 males in the high-dose group males had microscopic lesions in the kidney described as slight tubular cell swelling. There were no microscopic lesions in the kidney of mid-dose group F_1 males corresponding to the increased organ weight, therefore, the reviewer considers the organ weight change to be treatment related, but not toxicologically significant. Changes in brain, pituitary, and ovarian weights in high-dose group F_1 females were considered incidental and/or not toxicologically significant, because no associated microscopic pathology was observed in these animals.

Evaluation of reproductive performance showed no treatment-related effects on mating index in either male or female rats, fertility index, or gestation index. There was a significant increase in the number of implants/dam in high-dose group F_0 rats that corresponded to a slightly larger number of pups/litter in the same group. The slight increase in the number of pups/litter also was correlated with a slight, but significant decrease in weight of high-dose group F_1 pups on lactation day 0. Pup weight gain was not affected at any dose for either generation. Survival indices were not affected by treatment with the test material at any time during lactation for F_1 or F_2 pups.

Systemic NOAEL (males) = 100 ppm (4.85-13.75 mg/kg/day)
Systemic LOAEL (males) = 1000 ppm (50.0-138.7 mg/kg/day, HDT) based on kidney lesions (slight tubular cell swelling) in F_0 and F_1 male rats.
Systemic NOAEL (females) ≥1000 ppm (69.2-147.7 mg/kg/day, HDT)
Reproductive NOAEL ≥1000 ppm (50.1-138.7 mg/kg/day for males; 69.2-147.7 mg/kg/day for females, HDT)
Offspring NOAEL ≥1000 ppm (50-147.7 mg/kg/day, HDT)

C. STUDY DEFICIENCIES

No major deficiencies were noted for this study.

The reproductive study in the rats is classified **Acceptable/Guideline** and does satisfy the guideline requirement for a two-generation reproductive study [OPPTS 870.3800, (§83-4)] in the rat.

DATA EVALUATION RECORD

CYHALOFOP BUTYL
(XRD-537 BE)

STUDY TYPE: CHRONIC ORAL TOXICITY - DOG (OPPTS 870.4100 [§83-1])
MRID 45014708
Prepared for

Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
Jefferson Davis Highway
Arlington, VA 22202

Prepared by

Chemical Hazard Evaluation Group
Toxicology and Risk Analysis Section
Life Sciences Division
Oak Ridge National Laboratory
Oak Ridge, TN 37831
Task Order No.01-81CC

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Disclaimer

This review may have been altered subsequent to the contractor's signatures above.

Oak Ridge National Laboratory, managed by UT-Battelle, LLC., for the U.S. Department of Energy under contract number DE-AC05-00OR22725

CYHALOFOP BUTYL **Subchronic Oral Study-[OPPTS 870.4100 (83-1)]**

EPA Reviewer: John Whalan Date 5-15-01
Registration Action Branch 2 (7509C)
EPA Secondary Reviewer: SanYvette Williams-Foy, D.V.M. , Date 5/31/2K1
Registration Action Branch 2 (7509C)
TXR No: 0050348

DATA EVALUATION RECORD

STUDY TYPE: Chronic Oral Toxicity- Dog [OPPTS 870.4100 (§83-1)]

DP BARCODE: D268553 SUBMISSION CODE: S
P.C. CODE: 082583 TOX. CHEM. NO.: n/a

TEST MATERIAL: Cyhalofop butyl; purity ≥97%

SYNONYMS: R-(+)-n-Butyl-2-(4-(2-fluoro-4-cyanophenoxy)phenoxy)propionate; AGR 276541; propanoic acid, 2-(4-(4-cyano-2-fluorophenoxy)phenoxy)n-butyl ester (R(+));XRD-537nBu; XRD-537 BE

CITATION: Harada, T., et al. (1994). XRD-537 BE: 12-Month oral subchronic toxicity study in dogs. Institute of Environmental Toxicology, Suzuki-cho 2-772, Kodaira-shi, Tokyo 187, Japan. Laboratory Study ID GHF-P-1386. June 20, 1994. MRID 45014708.

SPONSOR: Dow Chemical Japan, Ltd., DowElanco Division, Seavans North 2-1, Shibaura 1-chrome, Minato-ku, Tokyo 105, Japan

EXECUTIVE SUMMARY: In a chronic toxicity study (MRID 45014708) XRD-537 BE (Lot no. AGR295713, purity 97.1%) was administered in diet to four dogs/sex at doses of 0, 50, 300, or 1800 ppm. Average doses to animals were 0, 1.22, 7.59, and 46.7 mg/kg/day for males, and 0, 1.29, 7.63, and 45.9 mg/kg/day for females.

No treatment-related mortality occurred during the study and the only clinical sign attributable to the test substance was loose stools during the first 13 weeks of the study in one male high-dose dog and one female high dose dog. No biologically or toxicologically relevant treatment-related effects were found on hematological, clinical chemistry, opthalomogic, or urinalysis parameters, and there were no effects on absolute or relative organ weights. Treatment with the test material did induce a slight decrease (12%) in the body weight of mid- and high-dose male dogs, as well as a statistically significant decrease of body weight gain. These effects were also observed from female dogs, but were of lesser intensity. These effects were not considered biologically or toxicologically relevant.

The gall bladders of 3/4 male and 3/4 female 1800 ppm dogs were distended and contained black sand-like material. This was also observed in 3/4 mid-dose female dogs. Microscopically, the gall bladders of 3/4 male and 1/4 female high-dose dogs contained areas of epithelial hyperplasia. PAS staining determined the hyperplastic effect was a result of increased mucous secretion. Distended gallbladders are common in laboratory dogs, however, because of the need to

use low fat feed. Hepatocellular cytoplasmic eosinophilia was observed in the livers of all male and 3/4 female high-dose dogs. In addition, one male high-dose dog was reported to have areas of centrilobular necrosis, but with absence of a hyperplastic response and without observable functional or architectural aberrations, the liver affects, though treatment-related, are not considered adverse.

Under the conditions of this study, the LOAEL for the systemic toxicity of XRD-537 BE in beagle dogs is undetermined. The NOAEL is ≥1800 ppm (46.7, and 45.9 mg/kg/day for males and females, respectively).

This chronic toxicity study is classified as **Acceptable/Guideline.** This study does satisfy the Subdivision F requirements for a chronic oral toxicity study in non-rodents [OPPTS 870.4100 (§83-1)].

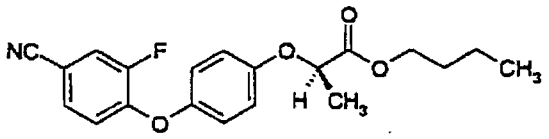
COMPLIANCE: Signed and dated Quality Assurance, GLP, Data Confidentiality, and Flagging statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: XRD-537 BE

   Description: off-white powder
   Lot/Batch #: AGR295713
   Purity: 97.1%
   Stability of compound: Stable in a cold and dark environment
   CAS #: 122008-85-9
   Structure:



2. Vehicle and/or positive control

   The test substance was administered in the diet. Control animals received food prepared in the same manner but lacking the test compound.

3. Test animals

   Species/strain: Beagle
   Age and weight at study initiation: approximately 5 months old, males 8.7 ± 0.7 kg, females 8.5 ± 0.9 kg
   Source: Suwa Farm of CSK Experimental Animal Laboratory Co., Ltd., Toyoda, Suwa-shi, Nagano.

Housing: individually in stainless steel cages
Diet: Certified diet DS (Oriental Yeast Co., Ltd., Azusawa, Itabashi-ku, Tokyo) 300 g/dog/day, moistened with 300 g water
Water: sterilized well water, *ad libitum*
Environmental conditions
Temperature: 24 ±2°C
Humidity: 55 ±10%
Air changes: 15 times per hour
Photoperiod: 12 hour light/dark cycle
Acclimation period: 30 days

B. STUDY DESIGN

1. In life dates

Start: 09/10/92; end: 09/20/93

2. Animal assignment

Dogs were assigned to the test groups in Table 1 by computer randomization based on body weight.

TABLE 1: Study design

Test group and dose level (ppm)		Animals per group		Average intake (mg/kg/day)	
		Male	Female	Male	Female
Control	0	4	4	0	0
Low	50	4	4	1.22	1.29
Mid	300	4	4	7.59	7.63
High	1800	4	4	46.7	45.9

Data taken from text table, p. 31, MRID 45014708.

3. Rationale for dose selection

Dose selection was based on the results of an earlier subchronic study (MRID 45014707) in which the lowest dose (100 ppm) produced no treatment-related effects and the highest dose induced organ weight changes (liver, thymus, and kidney) and histopathological alterations in the liver, thymus, kidney and thyroid.

4. Dose preparation and analysis

A premix was prepared by dissolving a specified amount of the test material in acetone then mixed with a portion of the ground diet. The premix was then blended with the remaining part of the feed by an SS-501 mixer to obtain the desired concentration and a homogeneous distribution of the test substance. The control diet was treated

with acetone and prepared in the same manner. A 30 minute period was allowed for evaporation of acetone before the prepared diets were sealed in plastic bags, placed in a plastic container, and stored at 4°C until used. The diets were prepared immediately before the study and at 3-4 week intervals thereafter. When in use, the diets were placed in aluminum containers and stored at room temperature for up to 15 days.

Stability: The stability of the test material in the diet was confirmed and reported in a previous study (MRID 45014707).

Homogeneity: Homogeneity of the test substance in the diet was monitored with 50 g samples taken from the top, middle, and bottom of the mixer at each dose level prior to treatment.

Concentration: Concentration of the test material at each dose level was checked with 50 g samples taken from the middle of each mixer prior to initiation of treatment.

Results –

Stability: In an earlier study (MRID 45014707), a 100 ppm diet was stored at 4°C for 59 days, room temperature for 15 days, and then for one day mixed 1:1 with water. Analysis of the samples showed the test material was present at 92-100% of the initial concentration.

Homogeneity: Homogeneity of the test material in samples from the top, middle, and bottom of each dose preparation was within 91-92% of the target concentration. The coefficient of variation for mean values at each dose level was within 2.5%.

Concentration: Average concentrations of the test substance in samples from the 50, 300, and 1800 ppm diets were 46, 276, and 1643 ppm, respectively. Mean values for all samples were within 91% of nominal dose levels.

Conclusion:

The test material was stable in the diet for the length of time required in the study. Concentrations of the test substance in all diet levels were acceptable and were homogeneously distributed.

5. Statistics

Treated groups were compared with controls by sex. Body weight, body weight gain, hematological and biochemical data, urine volume and specific gravity, and absolute and relative organ weights were analyzed by multiple comparison tests using Dunnett's or Scheffe's method. Significance was evaluated at the $p<0.05$ and 0.01 levels. The Mann-Whitney U test was used to evaluate food consumption data and other

urinalysis parameters. Fisher's exact probability test was used for clinical signs, mortality, ophthalmology, and pathology data.

C. METHODS

1. Observations

Animals were inspected at least once daily for signs of toxicity and mortality. A more detailed observation was conducted weekly.

2. Body weight

The dogs were weighed at the start of treatment, weekly for the first 13 weeks, at 16 weeks, and every 4 weeks thereafter for the remainder of the study.

3. Food consumption and food efficiency

Food consumption (g) for each animal was determined weekly. Food efficiency was not calculated.

4. Ophthalmoscopic examination

Ophthalmoscopic examinations were conducted on each dog prior to the start of the study and after 26 and 52 weeks of treatment. Using a direct ophthalmoscope, the eyeball, eyelid, conjunctiva, cornea, anterior chamber, pupil, iris, lens, vitreous body, and fundus were examined.

5. Blood

Blood samples were drawn from all dogs before the start of treatment, and at weeks 13, 26, and 52. Samples were collected from the cephalic vein of fasted dogs. The CHECKED (X) parameters were examined.

a. Hematology

X	Hematocrit (HCT)* (packed cell vol.)	X	Leukocyte differential count*
X	Hemoglobin (HGB)*	X	Mean corpusc. HGB (MCH)
	Methemoglobin	X	Mean corpusc. HGB conc. (MCHC)
X	Leukocyte count (WBC)*	X	Mean corpusc. volume (MCV)
X	Erythrocyte count (RBC)*		Reticulocyte count
X	Platelet count*		
	Blood clotting measurements*		
	(Thromboplastin time)		
	(Clotting time)		

Data taken from p. 16, MRID 45014708.

* Required for chronic studies based on Subdivision F Guidelines

b. Clinical chemistry

	ELECTROLYTES		OTHER
X	Calcium*	X	Albumin*
X	Chloride*	X	Creatinine*
	Magnesium	X	Blood urea nitrogen*
X	Phosphorus*	X	Total Cholesterol
X	Potassium*	X	Globulins
X	Sodium*	X	Glucose*
	ENZYMES	X	Total bilirubin
X	Alkaline phosphatase (ALK)	X	Total serum protein (TSP)*
X	Serum alanine aminotransferase (ALT)*		Osmolality
X	Serum aspartate aminotransferase (AST)*	X	Triglycerides
X	Gamma glutamyl transferase (GGT)	X	Albumin/Globulin ratio
X	Creatine Phosphokinase (CPK)		

Data taken from pp. 17-18, MRID 45014708.
* Required for chronic studies based on Subdivision F Guidelines

6. Urinalysis was conducted on all dogs prior to treatment, and at weeks 13, 26, and 52. Urine samples were collected over a 24 hour period for each animal. The CHECKED (X) parameters were examined.

X	Appearance*	X	Protein*
X	Volume*	X	Glucose*
X	Specific Gravity*	X	Ketones
X	pH*	X	Blood*
X	Sediment (microscopic)	X	Bilirubin
		X	Urobilinogen

Data taken from p. 15, MRID 45014708.
* Required for chronic studies based on Subdivision F Guidelines

7. Sacrifice and pathology

All animals were anesthetized with sodium pentobarbital, exsanguinated, and necropsied. Gross morphological examinations were conducted on all dogs and major tissues and organs. The checked (XX) organs were weighed. Organs checked X were sectioned and examined microscopically for all animals.

	DIGESTIVE SYSTEM		CARDIOVASC./HEMAT.		NEUROLOGIC
X	Salivary glands*	X	Aorta*	XX	Brain*+
X	Esophagus*	XX	Heart*+	XX	Pituitary*
X	Stomach*	X	Bone marrow*	X	Eyes
X	Intestines*	X	Lymph nodes* (mesenteric)	X	Nerve (sciatic)
X	Rectum*	XX	Spleen*+	X	Spinal Cord (cervical and lumbar)
XX	Liver*+	XX	Thymus*		
X	Gall bladder*				
X	Pancreas*		UROGENITAL		GLANDULAR
		XX	Kidneys*+	XX	Adrenal gland*+
	RESPIRATORY	X	Urinary bladder*	X	Harderian glands
X	Trachea*	XX	Testes*+	X	Mammary gland
X	Lung*+	XX	Ovaries*+	XX	Parathyroids*
		X	Prostate	XX	Thyroids*
		X	Uterus*		
		X	Oviduct*		OTHER
				X	Skeletal muscle
				X	All gross lesions and masses*
				X	Skin

Data taken from p. 19-21, MRID 45014708.
* Required for chronic studies based on Subdivision F Guidelines
+ Organ weight required in subchronic and chronic studies.

II. RESULTS

A. OBSERVATIONS

1. Toxicity

The only treatment related clinical sign observed was loose stools observed from one high dose male and one high dose female during the first 13 weeks of the study.

2. Mortality

There were no treatment-related deaths during the study.

B. BODY WEIGHT

Group mean body weights and body weight gains are shown in Table 2. The body weight of mid- and high-dose male dogs decreased ~12% from controls during the 52 weeks of the study, although the decrease was not statistically significant. Overall body weight gain of male mid- and high-dose dogs was decreased ~29% ($p \leq 0.05$). Although the overall body weight gain of female dogs of the mid-dose group was decreased ~19%, no statistically significant effects on the body weight of treated female dogs was found.

TABLE 2. Selected mean body weights (kg) and body weight gains (kg) of dogs fed XRD-537 BE for 52 weeks.

Study week	Dose level (ppm)			
	0	50	300	1800
Body Weight, Males				
Initial	8.7	8.7	8.6	8.7
1	9.0	9.1	8.9	9.0
13	12.1	11.7	11.3	11.1
28	13.3	12.7	12.0	11.8
52	14.6	13.5	12.8 (12)	12.8 (12)
Body Weight Gain, Males				
Week 0-1	0.30	0.33	0.30	0.28
0-13	3.35	2.93	2.65	2.35
0-28	4.60	4.00	3.40	3.10* (32)[a]
0-52	5.85	4.73	4.18* (29)	4.13* (29)
Females, Body Weight				
Initial	8.5	8.5	8.5	8.6
1	8.8	8.7	8.7	8.9
13	11.1	10.5	10.6	10.9
28	12.0	11.1	11.5	12.0
52	13.2	12.2	12.3 (7)	12.8 (3)
Body Weight Gain, Females				
Week 0-1	0.28	0.20	0.23	0.28
0-13	2.58	1.98	2.08	2.33
0-28	3.43	2.58	3.03	3.43
0-52	4.65	3.75	3.75 (19)	4.23 (9)

Data taken from Tables 5-8, pp. 57-60, MRID 45014708.
[a]Number in parenthesis is percent difference from control

C. FOOD CONSUMPTION and FOOD EFFICIENCY

1. Food consumption and food efficiency

Food consumption for males and females in all treated groups was comparable with controls throughout the study. Compound consumption is shown in Table 1. Food efficiency was not calculated.

D. WATER INTAKE

Water consumption was not measured.

E. OPHTHALMOSCOPIC EXAMINATION

No treatment-related visible lesions were found on any dog during the study.

F. BLOOD WORK

1. Hematology – No toxicologically or biologically significant effects were found.

2. Clinical chemistry – No toxicologically or biologically significant effects were found.

G. URINALYSIS

No toxicologically or biologically significant effects were found.

H. SACRIFICE AND PATHOLOGY

1. Organ weight – No toxicologically or biologically significant effects were found.

2. Gross pathology – The gall bladders of 3/4 high-dose males, 3/4 high-dose females, and 3/4 mid-dose females were distended and/or contained black sand-like contents. In addition, the livers of 3/4 high-dose males and the kidneys of 1/4 high-dose females were pale. One high-dose female had a thickened stomach mucosa and another high-dose female had consolidated foci and adhesions in all lobes of the lungs. No other significant treatment-related effects were found.

3. Microscopic pathology – Incidences of treatment-related microscopic changes found in dogs fed the test material for 52 weeks are shown in Table 3. Treatment with the test material induced hepatocellular cytoplasmic eosinophilia in all male high-dose dogs and 3/4 female high-dose dogs. This was not observed in any other treatment group. In conjunction with the eosinophilia, 1/4 high-dose male dogs had developed centrilobular necrosis. The study report does not state that hepatic hypertrophy was found. The gallbladders of 3/4 high-dose male dogs and 1/4 high-dose female dogs had developed epithelial hyperplasia. PAS (pararosaniline) staining showed that increased mucous secretion was responsible for the hyperplastic effect. One female dog was found to have foci and lung adhesions at necropsy that had resulted from involuntary diet aspiration.

TABLE 3. Incidence of treatment-related microscopic changes in dogs fed XRD-537 BE for 52 weeks.				
Dose (ppm)	0	50	300	1800
Males				
Liver				
Cytoplasmic eosinophilia	0	0	0	4*
Centrilobular necrosis	0	0	0	1
Gallbladder				
Epithelial hyperplasia	0	0	0	3
Females				
Liver				
Cytoplasmic eosinophilia	0	0	0	3
Gallbladder				
Epithelial hyperplasia	0	0	0	1

Data taken from Tables 25-26, pp. 117-119, MRID 45014708.
*Significantly different from control, $p<0.05$.

III. DISCUSSION

A. DISCUSSION

No treatment-related mortality occurred during the study and the only clinical sign attributable to the test substance was loose stools during the first 13 weeks of the study in one male high-dose dog and one female high dose dog. No biologically or toxicologically relevant treatment-related effects were found on hematological, clinical chemistry, ophthalomogic, or urinalysis parameters, and there were no effects on absolute or relative organ weights.

Treatment with the test material did induce a slight decrease (12%) in the body weight of mid- and high-dose male dogs, as well as a statistically significant decrease ($p \leq 0.05$) of body weight gain. These effects were also observed from female dogs, but were of lesser intensity. However, the reviewer feels that these slight differences from control are not biologically or toxicologically relevant.

At necropsy, the gall bladders of 3/4 male and 3/4 female high-dose dogs were distended and/or contained black sand-like material. This was also observed in 3/4 mid-dose female dogs. Microscopically, the gall bladders 3/4 male and 1/4 female high-dose dogs contained areas of epithelial hyperplasia. PAS staining determined the hyperplastic effect was a result of increased mucous secretion. Distended gallbladders are common in laboratory dogs because of the need to use low fat feed.

Hepatocellular cytoplasmic eosinophilia was observed in the livers of all male and 3/4 female high-dose dogs. In addition, one male high-dose dog was reported to have areas of centrilobular necrosis. Review of the liver plate provided in the study report from this

dog, shows the necrosis to be focal while the hepatic architecture was maintained. Therefore, in the absence of a hyperplastic response and without observable functional or architectural aberrations, the liver effects, though treatment-related are not considered adverse.

Under the conditions of this study, the LOAEL for the systemic toxicity of XRD-537 BE in beagle dogs is undetermined. The NOAEL is ≥1800 ppm (46.7, and 45.9 mg/kg/day for males and females, respectively).

This chronic toxicity study is classified as **Acceptable/Guideline.** This study satisfies the Subdivision F requirements for a chronic oral toxicity study in non-rodents [OPPTS 870.4100 (§83-1)].

B. STUDY DEFICIENCIES

None found.

DATA EVALUATION RECORD

CYHALOFOP BUTYL
(XRD-537 BE)

STUDY TYPE: CARCINOGENICITY (FEEDING) – MOUSE (OPPTS 870.4200 [§83-2])
MRID 45000418

Prepared for

Health Effects Division
Office of Pesticide Programs
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Prepared by

Chemical Hazard Evaluation Group
Toxicology and Risk Analysis Section
Life Sciences Division
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Task Order No. 01-81DD

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Disclaimer

This review may have been altered subsequent to the contractor's signatures above.

Oak Ridge National Laboratory, managed by UT-Battelle, LLC, for the U.S. Department of Energy under Contract No. DE-AC05-00OR22725

CYHALOFOP BUTYL, technical **Carcinogenicity [OPPTS 870.4200 (§ 83-2)]**

EPA Reviewer: John Whalan
Registration Action Branch 2 (7509C)
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Registration Action Branch 2 (7509C)
TXR No.: 0050348

John Whalan, Date 6-5-02
[signature], Date 6/5/02

DATA EVALUATION RECORD

STUDY TYPE: Carcinogenicity Feeding - Mouse [OPPTS 870.4200 (§83-2)]

DP BARCODE: D268553
P.C. CODE: 082583

SUBMISSION CODE: None
TOX. CHEM. NO.: None

TEST MATERIAL (PURITY): Cyhalofop butyl, technical (97.1%)

SYNONYMS: XRD-537 BE; R-(+)-n-butyl-2-(4-(2-fluoro-4-cyanophenoxy) phenoxy)propanoate

CITATION: Harada, T., Ebino, K., Odanaka, Y., Maita, K. (1994) XRD-537 BE: 18-month oral chronic toxicity and oncogenicity study in mice. The Institute of Environmental Toxicology, Mitsukaido Laboratories, 4321, Uchimoriya-cho, Mitsukaido-shi, Ibaraki 303, Japan. Laboratory Study GHF-P-1384, IET 90-0166, June 2, 1994. MRID 45000418. Unpublished.

SPONSORS: Dow Chemical Japan Ltd., DowElanco Division, Seavans North, 2-1 Shibaura 1-chome, Minato-ku, Tokyo 105, Japan; Nichimen Corporation, 11-1, Nihonbashi 3-chome, Chuo-ku, Tokyo 103, Japan.

EXECUTIVE SUMMARY: In a carcinogenicity study (MRID 45000418), XRD-537 BE (97.1% purity, lot no. AGR 295713) was administered to groups of 76 male and 76 female ICR (Crj:CD-1) mice in the diet at concentrations of 0, 3, 10, or 100 ppm. Fifty-two mice/sex/group comprised the main study and were administered the test compound for 78 weeks. The satellite group, composed of 24 mice/sex/group were sacrificed at interim times of 26 weeks (10 mice/sex/group) and 52 weeks (10 mice/sex/group). The dietary concentrations of 3, 10, and 100 ppm resulted in daily compound intake of 0.31, 0.99, or 10.06 mg/kg/day, respectively, for males, and 0.29, 0.99, or 10.28 mg/kg/day, respectively, for females.

Treatment with XRD-537 BE did not result in increased mortalities or increases in incidences of clinical signs in treated groups compared with the control groups. At the end of 78 weeks, there were no effects of treatment on body weight, food consumption, food efficiency, hematology, clinical chemistry parameters, or organ weights for either sex. Treated mice that died during the study were emaciated compared with respective control groups; this effect was not present in animals that survived to terminal sacrifice.

Increases in liver weight for both sexes, observed at the 26-week interim sacrifice, were not significant at the 78-week terminal sacrifice. Grossly enlarged, dark-colored livers with histological correlates of hepatocellular swelling with minute eosinophilic granules, observed in main study males and females in the 100 ppm group and males in the 10 ppm group after 78 weeks, were considered liver hypertrophy, an adaptive response to chemical administration. A 6% decrease in brain weight in all treated female mice at the 52-week sacrifice was transient and considered incidental to treatment. Treatment-related kidney lesions in female mice in the 100 ppm main study group consisted of tubular dilatation ($p<0.01$), chronic glomerulonephritis ($p<0.05$), and hyaline casts ($p<0.01$). In male mice these kidney lesions were either not observed or incidences were not dose-related. Incidences of mucosal epithelial hyperplasia of the glandular portion of the stomach were increased in male mice in the 100 ppm group ($p<0.01$).

The LOAEL for male and female mice is 100 ppm in the diet (males, 10.1 mg/kg/day; females, 10.3 mg/kg/day) based on effects on the kidney including tubular dilatation, chronic glomerulonephritis, and hyaline casts in females, and hyper-plasia of the stomach mucosal epithelium in males. The NOAEL for male and female mice is 10 ppm in the diet (approximately 1 mg/kg/day).

There was no evidence of carcinogenic potential under the conditions of this study. The doses used in this study were inadequate for assessing carcinogenicity.

This carcinogenicity study in the mouse is **Unceptable/Guideline** and does not satisfy the guideline requirement for an carcinogenicity study [OPPTS 870.4200 (§ 83-2)] in mice.

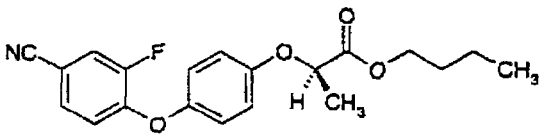
COMPLIANCE: Signed and dated GLP, Quality Assurance, Data Confidentiality, and Flagging statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: XRD-537 BE

   Description: Off-white powder
   Lot No.: AGR 295713
   Purity: 97.1%
   Stability of compound: stable for two years.
   CAS No.: 122008-85-9
   Structure:



2. Vehicle and/or positive control: The test material was dissolved in acetone and mixed with feed; the control diet was treated with acetone and prepared in the same manner.

3. Test animals:

Species: Mouse
Strain: ICR (Crj:CD-1)
Age and weight at study initiation: age: 5 weeks; average weight: males, 28.1±1.4 g; females, 22.9±1.2 g
Source: Charles River Japan, Inc, Atsugi Breeding Center, Shimofurusawa, Atsugi-shi, Kanagawa; Hino Breeding Center, Hino-cho, Gamoh-gun, Shiga
Housing: animals were housed four/cage in aluminum cages with wire mesh floors; the cages were placed in stainless steel racks with four tiers each. Cages were replaced and rotated every four weeks.
Diet: Certified diet MF Mash (Oriental Yeast Company), *ad libitum*
Water: Filtered, sterilized well water, *ad libitum*
Environmental conditions:
Temperature: 24±2°C
Relative humidity: 55±15%
Ventilation: air changes 15 times/hour
Light cycle: 12 hours light: 12 hours dark
Acclimation period: 9 days (males); 7 days (females)

B. STUDY DESIGN

1. In life dates: Start: October 17 and 25, 1991; end: April 15 and 23, 1993

2. Animal assignment

Animals whose body weights were ±20% of the mean weight and were free of clinical abnormalities were assigned to the test groups in Table 1 by means of a computerized randomization procedure.

TABLE 1: Study design

Test group	Dose Level (ppm)	Mean dose to animal (mg/kg/day)		Number of Animals			
		Male	Female	Male		Female	
				Main	Satellite[a]	Main	Satellite[a]
1 (control)	0	0	0	52	24	52	24
2 (low dose)	3	0.3112	0.2936	52	24	52	24
3 (mid dose)	10	0.986	0.989	52	24	52	24
4 (high dose)	100	10.06	10.28	52	24	52	24

Data taken from pp. 11 and 30, MRID 45000418.
[a]Ten animals/sex from the satellite group were selected for interim evaluations at 26 and 52 weeks.

3. Dose selection

Dietary concentrations were based on a previous subchronic study (IET 90-0164) in which groups of male and female mice were administered dietary levels of 0, 3, 30, 100, or 300 ppm. At necropsy, dark-colored livers were observed in both sexes at 100 and 300 ppm. Liver weights were increased in males receiving ≥30 ppm and in females administered 100 and 300 ppm. Kidney weights were increased in females receiving ≥30 ppm; hepatocellular swelling was observed in both males and females receiving ≥30 ppm. Urinalysis revealed significant decreases in pH and ketones in males at ≥30 ppm. Based on these results, 100 ppm was chosen as the high dose and was expected to result in systemic effects without substantially altering the life span.

4. Diet preparation and analysis

Diets were prepared prior to treatment and every 4 weeks thereafter. A premix was prepared by adding an appropriate amount of the test material, dissolved in acetone, to the basal diet. The dietary concentrations used in the study were prepared by diluting the premix with untreated diet in a mixer. The control diet was also treated with acetone and prepared in the same manner. Following a 30-minute period to allow the acetone to evaporate, the prepared test diets were sealed into plastic bags and stored in aluminum containers in the dark at 4°C. Animals were provided with fresh diet twice a week.

Prior to treatment, samples were taken from the top, middle, and bottom of the mixer for each dose level and analyzed for homogeneity. Samples taken from the middle of the mixer were analyzed for concentration at each preparation time (17 times). The stability and enantiomer ratio (R/S) of the test material in the diets were determined in a previous study (IET 90-0165). In that study, samples from a 600 ppm concentration were analyzed at the following time points: day 0, 42 days (following storage at 4°C in the dark), 57 days (following storage at 4°C in the dark for 42 days and then room temperature in the animal room for 15 days), and 64 days (following storage at 4°C in the dark for 42 days, room temperature in the animal room for 15 days, and exposed to air in a feeding jar for 7 days).

Results

Homogeneity – The ranges of concentrations of the test material in samples taken from different locations of the 3, 10, and 100 ppm dietary concentrations were 3.0-3.3, 9.3-9.8, and 86-94 ppm, respectively. Coefficients of variation were 5.4, 1.2, and 4.0%, respectively.

Stability – The enantiomer ratio following storage under the conditions outlined above was 98.9%/1.1% or 99.0%/1.0 at each time point. The R/S ratio claimed was 99.6%/0.4%.

Concentration analysis – Mean concentrations of the test substance from 17 sampling times for the 3, 10, and 100 ppm nominal concentrations were 3.0±0.19,

9.5±0.22, and 94±1.7 ppm. These concentrations were 100, 95, and 94% of nominal concentrations, respectively.

The analytical data indicated that the mixing procedure was adequate, that the variance between nominal and actual dosage to the animals was acceptable, and the test material was stable in the diet under storage conditions of the study.

5. Statistics

Body weight, food consumption, urine specific gravity, hematology , blood biochemistry, and organ weights were analyzed utilizing Dunnett's or Scheffe's method for multiple comparisons. Urine parameters (except specific gravity) were analyzed with the Mann-Whitney U test. Incidences of clinical signs, ophthalmology signs, and pathologic lesions were analyzed with Fisher's exact probability test. Statistical significance was flagged at $p<0.05$ or $p<0.01$. Statistical analyses were provided for total animals (main study and satellite animals) and animals sacrificed at 26, 52, and 78 weeks, but not for total main study animals (animals sacrificed at 78 weeks plus animals in the main study sacrificed *in extremis* or found dead).

C. METHODS

1. Observations

Animals in both the main and satellite groups were observed for mortality, morbidity, and clinical signs at least once a day. The date of onset, nature, severity, and duration of clinical signs were recorded. Detailed examinations including palpation for masses were performed once a week.

2. Body weight

All animals in both the main and satellite groups were weighed weekly for the first 13 weeks, once every 4 weeks from week 16 to study termination, and at necropsy.

3. Food consumption, compound intake, and food efficiency

Food consumption was calculated for animals in the main study. Food consumption was measured at the same time intervals as body weight measurements were taken: once a week during the first 13 weeks, once every 4 weeks from week 16 to study termination, and at necropsy. At each measurement interval, food consumption was measured over a 3-day period for each cage; this measurement was adjusted to a daily food consumption value per animal. Group mean values at each time period were reported as g/mouse/day. Chemical intake was calculated from the data on food consumption, nominal dietary level, and body weights. Food efficiency was calculated as: (g body weight gain/g food consumption) x 100.

4. Ophthalmoscopic examination

Ophthalmoscopic examinations were performed using a halogen ophthalmoscope on both main and satellite animals prior to treatment and on all surviving animals in the main control and high-dose groups at 78 weeks of treatment.

5. Blood was collected from the posterior vena cava of mice under ether anesthesia. Samples were taken from 10 mice/sex/dose of the satellite group at 26 and 52 weeks (9 male mice in the 100 ppm group at 52 weeks) and from 10 animals/sex/group of main group mice at 78 weeks. The CHECKED (X) parameters were examined.

a. Hematology

X		X	
X	Hematocrit (HCT)	X	Leukocyte differential count*
X	Hemoglobin (HGB)	X	Mean corpuscular HGB (MCH)
X	Leukocyte count (WBC)	X	Mean corpuscular HGB concentration (MCHC)
X	Erythrocyte count (RBC)	X	Mean corpuscular volume (MCV)
X	Platelet count		Reticulocyte count
	Blood clotting measurements		
	(Thromboplastin time)		
	(Clotting time)		
	(Prothrombin time)		

* Minimum required for oncogenicity studies unless effects are observed, based on OPPTS 870.4200 Guidelines.

b. Clinical chemistry

The CHECKED (X) serum chemistry parameters were examined.

X		X	
X	Calcium	X	Total protein
X	Glucose	X	Total cholesterol
X	Urea nitrogen	X	Triglycerides
X	Albumin	X	Alkaline phosphatase
X	Globulin	X	Glutamic oxaloacetic transaminase
X	Albumin/globulin ratio	X	Glutamic pyruvic transaminase

6. Urinalysis*

Urinalysis tests were conducted on 10 satellite mice/sex/group at 26 and 52 weeks and on 10 randomly selected mice/sex/group from the main group at 78 weeks. Fresh urine was obtained by pressing on the lower abdominal region of each mouse. The CHECKED (X) parameters were examined.

X		X	
	Appearance	X	Glucose
	Volume	X	Ketones
X	Specific gravity		Bilirubin
X	pH	X	Blood
	Sediment (microscopic)		Nitrites
X	Protein	X	Urobilinogen

*Not required for carcinogenicity studies by OPPTS 870.4200 Guidelines.

7. Sacrifice and pathology

Necropsies were performed on all animals sacrificed in the satellite groups, on all animals in the main group at terminal sacrifice, and on all animals in the main group that died or were killed at unscheduled times during the treatment period. At the study terminations (26, 52, and 78 weeks), all animals were anesthetized with ether and sacrificed by exsanguination from the posterior vena cava. The CHECKED (X) tissues from all groups were collected for histopathological examination and were fixed in 10% neutral-buffered formalin. Preparations were stained with hematoxylin and eosin. The (XX) organs from all animals were weighed.

X	DIGESTIVE SYSTEM	X	CARDIOVASC./HEMAT.	X	NEUROLOGIC
	Tongue	X	Aorta*	XX	Brain*+
	Oral tissue	XX	Heart*	X	Peripheral. nerve*
X	Salivary glands*	X	Bone marrow*	X	Spinal cord (3 levels)*
X	Esophagus*	X	Lymph nodes*	X	Pituitary*
X	Stomach*	XX	Spleen*	X	Eyes*
X	Duodenum*	X	Thymus*		
X	Jejunum*				GLANDULAR
X	Ileum*		UROGENITAL	XX	Adrenal gland*
X	Cecum*	XX	Kidneys*+		Lacrimal/Harderian glands
X	Colon*	X	Urinary bladder*	X	Mammary gland*
X	Rectum*	XX	Testes*+	X	Parathyroids*
XX	Liver*-	X	Epididymis	X	Thyroids*
X	Gall bladder*	X	Prostate		Zymbal's gland
X	Pancreas*	X	Seminal vesicle		
		X	Coagulating gland		OTHER
	RESPIRATORY		Preputial gland	X	Bone*
X	Trachea*	X	Ovaries*+	X	Skeletal muscle*
X	Lung*	X	Uterus*	X	Skin*
	Nose	X	Cervix		Mediastinal tissue
	Pharynx		Oviduct		Mesenteric tissue
	Larynx		Vagina	X	All gross lesions and masses*

* Required for oncogenicity studies based on OPPTS 870.4200 Guidelines.
+ Organ weight required in oncogenicity studies.

II. RESULTS

A. OBSERVATIONS

1. Toxicity

The following clinical signs were recorded during daily observations. In the main group, the incidences of emaciation in male mice in the 3 and 10 ppm groups (both 11/52) were significantly higher ($p<0.01$) than in the control group (1/51) and the incidences of tactile hair loss (6/52) and dermal wounds (20/52) in male mice in the 3 ppm group were significantly higher than in the control group (0/51 and 11/51, respectively; both $p<0.05$). The incidence of emaciation in male mice in the 100 ppm group was 6/52 (not statistically significant). These clinical signs were not observed in female mice.

2. Mortality

The survival rates of mice in the main group were not affected by treatment (Table 2). One male mouse in the control group died in an accident during week 36.

TABLE 2. Survival (percent) of male and female mice fed XRD-537 BE for 78 weeks (main group)

Dietary concentration (ppm)			
0	3	10	100
Males			
38/51 (75)[a]	35/52 (67)	34/52 (65)	42/52 (81)
Females			
39/52 (75)	35/52 (67)	36/52 (69)	40/52 (77)

Data taken from p. 29, MRID 45000418.
[a]Number in parentheses represents % of initial number.

B. BODY WEIGHT

Group mean body weights of male and female mice in the main group for selected weeks are summarized in Table 3. Body weights were comparable among groups for each sex throughout the study.

TABLE 3. Group mean body weights and weight gains of main group male and female mice fed XRD-537 BE in the diet for 78 weeks (g)

Week of treatment	Dietary concentration (ppm)			
	0	3	10	100
Males				
Body weight, week 0	28.1 ± 1.4[a]	28.1 ± 1.4	28.1 ± 1.4	28.1 ± 1.4
Body weight, week 52	51.7 ± 5.6	48.8 ± 6.3	50.7 ± 6.1	50.7 ± 7.0
Body weight, week 78	50.6 ± 5.2	50.9 ± 7.0 (101)[b]	48.6 ± 7.0 (96)	49.4 ± 7.0 (98)
Body weight gain, 0-78[c]	22.5	22.8 (101)	20.5 (91)	21.3 (95)
Females				
Body weight, week 0	22.9 ± 1.2	22.9 ± 1.2	22.9 ± 1.3	22.9 ± 1.2
Body weight, week 52	51.2 ± 6.2	50.5 ± 5.7	51.0 ± 8.0	49.5 ± 8.4
Body weight, week 78	50.1 ± 6.3	48.8 ± 7.3 (97)	51.7 ± 8.6 (103)	49.3 ± 8.6 (98)
Body weight gain, 0-78[c]	27.2	25.9 (95)	28.8 (106)	26.4 (97)

Data taken from Tables 5 and 6, pp. 87-92, MRID 45000418.
[a]Mean ± Standard deviation
[b]Numbers in parenthesis represent % difference from the control; calculated by the reviewer.
[c]Calculated by reviewer.

C. FOOD CONSUMPTION AND COMPOUND INTAKE

1. Food consumption

Mean daily food consumption did not differ among groups in the main study for either sex. Food intake was slightly, but significantly, increased for male mice in the 100 ppm group during week 1 and for female mice in the 100 ppm group during weeks 5, 11, and 60. Average food consumption values for males in the 0, 3, 10, and 100 ppm groups were 4.7, 4.9, 4.7, and 4.8 g/mouse/day, respectively. Average food consumption values for female mice in the 0, 3, 10, and 100 ppm groups were 4.2, 4.2, 4.3, and 4.4 g/mouse/day, respectively.

2. Compound consumption

The compound consumption was calculated based on feed consumption and body weight data. Doses are presented in Table 1.

3. Food efficiency

Food efficiency did not differ among groups for either sex in the main group. Overall food efficiency percentages for males in the 0, 3, 10, and 100 ppm groups were 3.7, 3.9, 4.0, and 3.8%, respectively. Overall food efficiency percentages for female mice in the 0, 3, 10, and 100 ppm groups were 3.4, 3.3, 3.4, and 3.4%, respectively.

D. BLOOD WORK

1. Hematology

Mean corpuscular hemoglobin concentration was significantly lower in the males in the 100 ppm group ($p<0.05$) after 26 weeks of treatment. Values in the 0, 3, 10, and 100 pm groups were 33.5, 33.0, 33.0, and 32.5 g/dl, respectively. A significantly lower mean corpuscular hemoglobin (MCH) value in female mice in the 10 ppm group after 26 weeks of treatment ($p<0.05$) was not dose-related.

2. Clinical chemistry

At the 52 week interim sacrifice there was a significant decrease in globulin (control, 2.71 g/dl; 100 ppm, 2.24 g/dl; $p<0.05$) and a significant increase in the albumin/ globulin ratio (control, 0.89; 100 ppm, 1.19; $p<0.01$) in male mice in the 100 ppm satellite group. These changes were not present in male mice at the 26 or 78 week observation period or in female mice at any observation period. No other significant changes in clinical chemistry parameters were observed in either sex.

E. URINALYSIS

Slightly higher urine pH values were noted for male mice in the 10 and 100 ppm groups at 26 weeks. pH values ranged up to 8.0 (1/10 males in the 10 ppm group and 4/10 males in the 100 ppm group; both $p<0.01$), whereas the highest value in the control group was 7.0. This difference was not noted at 52 or 78 weeks and was not noted in female mice at any time.

F. SACRIFICE AND PATHOLOGY

1. Organ weight

The body weights at interim and terminal sacrifices and the corresponding absolute and relative (to body weights) organ weights are summarized in Table 4. In males in the 100 ppm group, absolute and relative liver weights were increased at the 26-week interim sacrifice, by 24 and 19%, respectively (both, $p<0.01$), but increases were no longer statistically significant at the 52 week and terminal sacrifice. In females in the 100 ppm group, only the relative liver weight was increased, by 13% over the control weight ($p<0.05$) at the 26 week interim sacrifice. Neither absolute nor relative liver weights of females were increased at the 52 and 78 week sacrifices.

At the 52 week interim sacrifice, the absolute weight of the brain in all treated female groups was significantly lower than the control value (all, $p<0.05$), but the relative weight was comparable to controls. The magnitude of decrease was similar in all treated groups (6%) and did not appear to be dose related. This finding was not observed at other sacrifice times or in males at any observation time (data not presented in Table 4).

TABLE 4. Group mean final body weights (g) organ weights (g), and organ weights relative to body weight (%) in male and female mice fed XRD-537 BE for 26, 52 and 78 weeks

Treatment period, body weight, organ weight	Dietary concentration (ppm)			
	0	3	10	100
Males				
26 Week interim sacrifice				
Body weight	47.5 ± 6.0[a,b]	50.7 ± 3.8	47.7 ± 7.1	49.5 ± 6.7
Liver, absolute weight	2.24 ± 0.32	2.36 ± 0.24	2.38 ± 0.32	2.77 ± 0.44** (24)
Liver, relative weight	4.75 ± 0.61	4.66 ± 0.38	5.02 ± 0.44	5.64 ± 0.81** (19)
52 Week interim sacrifice				
Body weight	51.7 ± 7.5	52.5 ± 4.3	53.8 ± 6.4	53.3 ± 2.8
Liver, absolute weight	2.64 ± 0.44	2.71 ± 0.70	2.70 ± 0.26	3.14 ± 0.51
Liver, relative weight	5.11 ± 0.50	5.16 ± 1.26	5.04 ± 0.46	5.88 ± 0.69
Brain, absolute weight	0.523 ± 0.018	0.537 ± 0.035	0.517 ± 0.023	0.521 ± 0.016
78 Week terminal sacrifice				
Body weight	51.2 ± 3.5	50.6 ± 6.5	47.0 ± 7.0	53.0 ± 8.7
Liver, absolute weight	2.95 ± 1.15	3.23 ± 1.36	3.02 ± 1.00	3.35 ± 0.72
Liver, relative weight	5.80 ± 2.36	6.62 ± 3.48	6.42 ± 1.56	6.34 ± 0.85
Females				
26 Week interim sacrifice				
Body weight	44.3 ± 6.9	45.0 ± 7.0	46.6 ± 6.1	44.5 ± 5.2
Liver, absolute weight	1.81 ± 0.18	1.82 ± 0.24	1.87 ± 0.27	2.08 ± 0.32
Liver, relative weight	4.12 ± 0.39	4.05 ± 0.25	4.03 ± 0.39	4.67 ± 0.55* (13)
52 Week interim sacrifice				
Body weight	51.5 ± 7.9	56.7 ± 10.1	46.3 ± 8.3	49.8 ± 5.5
Liver, absolute weight	2.19 ± 0.35	2.42 ± 0.57	2.03 ± 0.46	2.22 ± 0.40
Liver, relative weight	4.28 ± 0.51	4.34 ± 1.13	4.41 ± 0.72	4.46 ± 0.61
Brain, absolute weight	0.551 ± 0.014	0.516 ± 0.030*	0.520 ± 0.033*	0.518 ± 0.024*
78 Week terminal sacrifice				
Body weight	49.1 ± 7.7	50.3 ± 7.2	51.3 ± 5.4	49.7 ± 11.1
Liver, absolute weight	2.32 ± 0.47	2.44 ± 0.33	2.24 ± 0.48	2.45 ± 0.37
Liver, relative weight	4.82 ± 1.15	4.94 ± 0.97	4.38 ± 0.75	5.08 ± 0.92

Data taken from Tables 23 and 24, pp. 159-170, MRID 45000418.
[a]Based on 10 animals/sex/group.
[b]Mean ± standard deviation.
*$p < 0.05$, significantly different from the control.
**$p<0.01$, significantly different from the control.

2. Gross pathology

Macroscopic findings are summarized in Table 5. Emaciation was observed in male and female mice at the terminal sacrifice and in mice killed *in extremis*/found dead. Emaciation was not observed in either sex at the 26 or 52 weeks interim sacrifices. For males, incidences of emaciation at the terminal sacrifice for the 0, 3, 10, and 100 ppm groups were 0/38, 3/35, 5/34 ($p<0.05$), and 3/42, respectively. Respective incidences in males found dead or killed *in extremis* were 1/13, 7/17 ($p<0.05$), 6/18,

and 4/10. Total incidences of emaciation in males, reported in Table 5, indicate that statistical significance was attained in all treatment groups. For females, incidences of emaciation at the terminal sacrifice for the 0, 3, 10, and 100 ppm groups were 0/39, 2/35, 0/36, and 3/40, respectively. Respective incidences for females found dead or killed *in extremis* were 0/13, 8/17 ($p<0.01$), 6/16 ($p<0.05$), and 4/12 ($p<0.05$). Total incidences in females, reported in Table 5, indicate that statistical significance was attained in all treatment groups.

A thickened wall of the glandular portion of the stomach was observed in male mice in the 100 ppm group only at the 78 week terminal sacrifice (controls, 1/38; 100 ppm, 7/42; $p<0.05$). This lesion was not observed in male mice at the interim sacrifices or in male mice sacrificed *in extremis*/found dead. Therefore, the total incidences for controls and the 100 pm group were 1/71 and 7/71 ($p<0.05$), respectively. Statistics were not provided for the main study animals (1/52 vs 7/52). In female mice, this lesion was observed only in the control group at the terminal sacrifice (3/39) and in one mouse in the 3 ppm group sacrificed killed *in extremis*.

Total incidences of dark-colored livers were significant in both males (25/71; $p<0.01$) and females (13/71; $p<0.01$) in the 100 ppm group. This finding was observed in 7, 9, and 9 male mice at the 26 and 52 week interim sacrifices and the 78-week terminal sacrifice, respectively. In females, incidences at the respective times were 7, 0, and 5; a dark-colored liver was also observed in one female in this group that was found dead or sacrificed *in extremis*. Livers in male mice in the 10 and 100 ppm groups were grossly enlarged at the 26-week interim sacrifice, 5/10 ($p<0.05$) and 7/10 ($p<0.01$) compared with 0/10 and 0/10 in the control and 3 ppm groups, respectively, but incidences of gross enlargement in all groups were 0 at the 52 week interim sacrifice and no longer statistically significant at the 78 week sacrifice.

TABLE 5. Macroscopic findings in male and female mice fed XRD-537 BE in the diet for 78 weeks

Finding or organ/tissue lesion	Dietary concentration (mg/kg/day)			
	0	3	10	100
Males				
Emaciation				
main study[a]	1/51[b,c]	10/52	11/52	7/52
totals[d]	1/71	10/72**	11/72**	7/71*
Stomach, thickened glandular wall				
main study	1/51	2/52	3/52	7/52
totals	1/71	2/72	3/72	7/71*
Liver, dark in color				
26-week interim sacrifice	0/10	0/10	1/10	7/9**
52-week interim sacrifice	0/10	0/10	2/10	9/9**
main study	0/51	0/52	0/52	9/52
totals	0/71	0/72	3/72	25/71**
Females				
Emaciation				
main study	0/52	10/52	6/52	7/52
totals	0/72	10/72**	6/72*	7/72**
Stomach, thickened glandular wall				
main study	3/52	1/52	0/52	0/52
totals	3/72	1/72	0/72	0/72
Liver, dark in color				
26-week interim sacrifice	0/10	0/10	0/10	7/10**
52-week interim sacrifice	0/10	0/10	0/10	0/10
main study	0/52	0/52	0/52	6/52
totals	0/72	0/72	0/72	13/72**

Data taken from the Tables 21 and 22, pp. 137-158, MRID 45000418.
[a]Mice at terminal sacrifice plus mice killed *in extremis*/found dead; statistics were not provided for the main study data.
[b]Number of mice with lesions/total number of mice examined.
[c]One animal died accidentally during week 36.
[d]Data includes animals sacrificed at 26, 52, and 78 weeks and animals killed *in extremis* or found dead.
*$p<0.05$, significantly different from control.
**$p<0.01$, significantly different from control.

3. Microscopic pathology

a. Non-neoplastic

Selected microscopic findings totaled from mice in the satellite and main study groups are summarized in Table 6. In the main study, incidences of hepatocellular swelling with minute eosinophilic granules were significantly increased over control values in male mice in the 100 ppm group (26/52; $p<0.01$) and in female mice in the 100 ppm group (12/52; $p<0.01$). In males in the 100 ppm group, the presence of this lesion was high at the 26 and 52 week interim sacrifices (10/10 and

9/9, respectively), with the percentage decreasing with time. This change was also present in males in the 10 ppm group at earlier interim sacrifice times, although in lower incidences compared with the 100 ppm group. Compared with males in the 100 ppm group, percentages of females in the 100 ppm group with this lesion were lower at the 26, 52, and 78-week interim sacrifice (4/10, 2/10, and 9/40 ($p<0.0.1$), respectively) and, in contrast to males, this finding was not observed in the 10 ppm treatment groups. Slight microgranuloma was significantly increased in female mice in the 10 and 100 ppm groups sacrificed at 78 weeks [incidences in order of ascending dose: 5/39, 9/35, 12/36 ($p<0.05$), and 14/40 ($p<0.05$); no incidences in female mice killed *in extremis* or found dead], but not when total mice were considered (incidences in order of ascending dose: 9/72,10/72, 13/72, and 15/72). This lesion was observed at low incidences in the female control and treated groups (0/10-2/10) at the earlier sacrifices. Incidences of microgranuloma were not dose-related in male mice. Centrilobular hepatocellular fatty changes were significantly decreased in male mice in the 100 ppm group ($p<0.01$) at the 78-week sacrifice; this lesions was not observed in female mice.

Kidney lesions were observed in female mice, primarily in the 100 ppm group. In the main study, incidences of chronic glomerulonephritis (12/52; $p<0.05$), tubular dilatation (10/52; $p<0.01$), and hyaline casts (16/52; $p<0.01$) were significantly higher compared with incidences in the control group (4/52, 1/52, and 4/52, respectively). Hyaline casts were also noted in the 3 ppm group of females. Most of these changes were not observed until the 78 week terminal sacrifice (casts) or in animals killed *in extremis* or found dead (tubular dilatation and chronic glomerulonephritis). Therefore, data for the interim sacrifices are not presented in Table 6. It should be noted that early changes of chronic glomerulonephritis were slightly lower in treated females than in controls so that incidences of early changes of chronic glomerulonephritis combined with incidences of chronic glomerulonephritis did not show a dose-response relationship (data not shown). These kidney lesions were not observed (tubular dilatation) or did not attain statistical significance (chronic glomerulonephritis and hyaline casts) in male mice.

Increased extramedullary hematopoiesis was observed in spleens of a significantly greater number of male mice in the 100 ppm group ($p<0.01$) as compared with controls, primarily in main study animals (0/10-2/10 incidence data not presented for interim sacrifices in Table 6). Incidences were approximately doubled over the control value in the 3 and 10 ppm groups of males, and attained significance in the 3 ppm group ($p<0.05$), but not in the 10 ppm group. Severity was generally slight in animals sacrificed on schedule and severe in animals found dead. Incidences in treated females were comparable to the control group.

TABLE 6. Non-neoplastic histopathology findings in male and female mice fed XRD-537 BE for 78 weeks

Organ/lesion	Dose (ppm)			
	0	3	10	100
Males				
Liver, hepatocellular swelling, minute eosinophilic granules				
26-week interim sacrifice	0/10[a]	0/10	3/10	10/10**
52-week interim sacrifice	0/10	0/10	6/10**	9/9**
main study[b]	0/51	0/52	1/52	26/52‡
Kidney				
Tubular dilatation (main study)	0/51	1/52	0/52	0/52
Chronic glomerulonephritis (main study)	0/51	3/52	3/52	4/51
Hyaline casts (main study)	4/51	4/52	4/52	8/51
Spleen				
Increased extramedullary hematopoiesis (main study)	9/51	17/52†	14/52	22/52‡
Adrenal, increased brown pigment, cortico-medullary junction				
main study	12/51	8/52	9/52	4/52†
Stomach, mucosal epithelial hyperplasia				
26-week interim sacrifice	3/10	2/10	0/10	3/10
52-week interim sacrifice	4/10	1/10	1/10	1/9
main study	9/51	14/52	13/52	21/52‡
Females				
Liver, hepatocellular swelling, minute eosinophilic granules				
26-week interim sacrifice	0/10	0/10	0/10	4/10*
52-week interim sacrifice	0/10	0/10	0/10	2/10
main study	0/52	0/52	0/52	12/52‡
Kidney				
Tubular dilatation (main study)	1/52	6/52	0/52	10/52‡
Chronic glomerulonephritis (main study)	4/52	6/52	5/52	12/52†
Hyaline casts (main study)	4/52	13/52†	9/52	16/52‡
Spleen				
Increased extramedullary hematopoiesis (main study)	7/52	12/52	9/52	5/52
Adrenal, increased brown pigment, cortico-medullary junction				
main study	4/52	8/52	12/52†	11/52†
Stomach, mucosal epithelial hyperplasia (main study)	4/52	1/52	3/52	3/52

Data taken from Table 27, pp. 186-192 and Table 28, pp. 207-213; MRID 45000418.
[a]Number of mice with lesions/total number of mice examined.
[b]Mice at 78-week terminal sacrifice plus mice killed *in extremis*/found dead.
*p<0.05, significantly different from control.
**p<0.01, significantly different from control.
†p<0.05, significantly different from control; calculated by reviewer.
‡p<0.01, significantly different from control; calculated by reviewer.

In the main study, the incidences of increased brown pigment in the cortico-medullary junction of adrenal glands were significantly higher in females in the 10 and 100 ppm groups (12/52 and 11/52, both p<0.05) as compared with controls, but the incidence was significantly lower in males in the 100 ppm group (4/52, p<0.05) as compared with male controls (12/51). Although present in low

numbers at the 26 and 52 week interim sacrifices, this lesion was most prevalent in females in the 10 and 100 ppm groups at the 78 week terminal sacrifice.

Incidences of hyperplasia of the epithelial mucosa of the glandular stomach were significantly increased in male mice in the 100 ppm main group (controls, 9/51; 100 ppm, 21/52; $p<0.01$), but the incidence of this lesion was not statistically significant compared with the control group when all animals and all sacrifice times were considered (incidences of 16/71, 17/72, 14/72, and 25/71 in the 0, 3, 10, and 100 ppm groups, respectively). This lesion was present in low , non-dose related numbers of males at the interim sacrifices. Females were not affected; total incidences in the main study ranged from 1/52-4/52.

The incidences of mineralization of the testis were decreased in all treated male mice, attaining statistical significance in the 100 ppm group. The lack of mineralization is not an adverse effect and data are not presented in Table 6.

Other changes that attained statistical significance were not dose-related.

b. Neoplastic

Various types of neoplasms were observed in all treated groups of male and female mice, but there were no significant differences between any treated group and the respective control group. The most common lesions in male mice were hepatocellular adenomas, present in 17/71 control males and 20/71 high-dose males, followed by lung adenomas and adenocarcinomas. Incidences of hepatocellular adenomas were low in female mice (control, 1/72; 100 ppm, 3/72); incidences of lung adenomas and adenocarcinomas were similar to those in males. Malignant lymphomas were also present in female mice at incidences of ≤14%. No specific types of tumors showed early onset.

III. DISCUSSION

A. INVESTIGATOR'S CONCLUSION

The investigators concluded that treatment with XRD-537 BE at concentrations of 0, 3, 10, or 100 ppm in the diet for 78 weeks had no effect on mortality rates of male and female mice. Emaciation was not considered of toxicological significance as it was observed principally in animals that were found dead or moribund and was not dose related.

The study authors stated that treatment with XRD-537 BE at 100 ppm in the diet compared with the controls resulted in grossly enlarged, dark-colored livers; increased relative liver weights in both sexes at 26 weeks and increased absolute liver weight in males at 26 weeks; hepatocellular swelling with minute eosinophilic granules in both sexes at 78 weeks; microgranuloma in the liver of females at the 78 week sacrifice;

and epithelial hyperplasia of the glandular stomach in males sacrificed by design after 78 weeks.

Treatment with 10 ppm resulted in enlarged livers in males at 26 weeks; hepatocellular swelling with minute eosinophilic granules in males but not in females; and an increased incidence of hepatic microgranuloma in females at the 78 week sacrifice (but not in all females combined).

There were no treatment-related changes in either sex administered 3 ppm in the diet. Therefore, the study authors considered 3 ppm a "maximum no-effect level," 10 ppm was considered a "minimum toxic level," and 100 ppm was considered a "sure toxic level."

The study investigators concluded that there was no evidence of carcinogenic activity for ICR (Crj:CD-1) mice of either sex administered diets containing XRD-537 BE at concentrations of 3, 10, or 100 ppm for 78 weeks.

B. REVIEWER'S DISCUSSION

There were no dose-related clinical signs in mice fed the test material at concentrations of 3, 10, or 100 ppm in the diet for up to 78 weeks. At the end of 78 weeks, there were no significant treatment-related effects on survival, body weight, food consumption, food efficiency, hematology, clinical chemistry parameters, or organ weights. Treatment-related effects in the 100 ppm group included lower mean corpuscular hemoglobin concentration in male mice at the 26 week interim sacrifice, a decrease in globulin and an increase in the albumin/globulin ratio in male mice at the 52 week interim sacrifice, increased liver weights at 26 weeks in both sexes, and decreased brain weight in females at 52 weeks. Incidences and organ weight changes did not attain statistical significance at 78 weeks and were, therefore, considered transient effects or toxicologically non-significant.

At autopsy, gross examination revealed emaciation in all treated groups of both sexes, but primarily in mice that died or were found moribund. The reviewer agrees with the study authors that this observation is of no or questionable toxicological significance because incidences were not clearly dose related and food consumption, food efficiency, body weights, and survival were not affected over the course of the study.

Mucosal epithelial hyperplasia of the glandular portion of the stomach appeared to be accelerated and more severe in aging male mice, with statistical significance attained in the 100 ppm group in the main sacrifice group. This lesion correlated with the thickened wall of the glandular stomach which could be observed grossly. This lesion indicates a treatment-related irritant effect of the test material in male mice. Incidences were low and similar in female control and treated groups. The study authors note that this lesion occurs with a high frequency in aged animals and was not present at the shorter time periods. The lesion is treatment related, but incidences were low, onset was late in the study, it occurred in only one sex, and it is a common

lesion of aging mice. Thus, the reviewer considers this lesion a minimal adverse effect.

Although statistical significance for increases in absolute and relative liver weights were not attained in any treatment group of either sex by the end of the study (indicating minimal hypertrophy), the livers in the treated groups were described as grossly enlarged and dark colored; microscopically, hepatocellular swelling with minute eosinophilic granules was observed. These treatment-related findings are consistent with liver hypertrophy which is an adaptive response to chemical intake. Liver hypertrophy may be accompanied by cell inclusions, in this case eosinophilic granules, the composition of which is unknown. Focal granuloma, also observed in female mice, is a common incidental finding in the liver of aging mice. Severity of this lesion in the female 100 ppm group was slight. Focal hepatocellular necrosis was not increased in treated mice and there were no corresponding enzyme, hematology or clinical chemistry changes, indicating that the function of the liver was not impaired. Therefore, the reviewer considers the effects on the liver an adaptive response rather than a toxicologically significant response. The reason for dark colored livers is unknown, but this observation may be an artifact of sacrifice.

Kidney lesions were confined to female mice in the 100 ppm group and consisted of tubular dilatation, chronic glomerulonephritis, and increased incidences of hyaline casts. While these findings were significantly higher for all females combined compared with the control group, the tubular dilatation and glomerulonephritis were confined primarily to females killed *in extremis* or found dead. Incidences were not significantly elevated in females at the 78 week terminal sacrifice. Furthermore, incidences of early changes of chronic glomerulonephritis were slightly lower in the treated female groups than in the control group, indicating that progression to the chronic state was either slightly accelerated in the higher dose groups or was related to death from other causes. Although found primarily in dead or moribund females in the 100 ppm group, survival in this group was not compromised compared with controls, and therefore the effect may be incidental to treatment. There were no related effects on clinical chemistry or urinary parameters in females. The toxicological significance of tubular dilatation and hyaline (protein) casts is unknown. Protein casts are usually associated with nephropathy in male rats and mice. Although some of the kidney lesions in the 100 ppm group may have been incidental to treatment, taken together, the lesions indicate a treatment-related toxicological effect. The significantly increased incidence of hyaline casts in the 3 ppm group was not considered treatment related as incidences were slightly lower in the 10 ppm group.

Although the incidence of increased brown pigment in the cortico-medullary junction of the adrenal gland was significantly elevated in female mice in the 10 and 100 ppm groups, particularly at 78 weeks, this lesion was significantly lower in male mice in the 100 ppm group compared with male controls. Pigmentation deposition in the cortico-medullary junction of the adrenal gland may be related to aging processes. This lesion was not accompanied by fatty deposition in the cortico-medullary

junction. Therefore, the toxicological significance of deposition of brown pigment in the adrenal gland is unknown.

Likewise, extramedullary hematopoiesis of the spleen was increased in only one sex (males), primarily at the 78 week sacrifice and in males found dead or moribund during the study. Statistical significance was attained in the 3 ppm group ($p<0.05$) and 100 ppm group ($p<0.01$) but not in the 10 ppm group (indicating a poor dose-response relationship). The proportion of animals with mild, moderate, and severe splenic hematopoiesis was similar among the control and treated groups. Mild hematopoiesis of the bone marrow was observed in many of the same animals. Hematology parameters were not affected and urobilinogen in the urine was not increased in the treated groups. Extramedullary hematopoiesis of the spleen is observed in aging mice. The fact that incidences were increased in the treated groups may be incidental to treatment.

The LOAEL for male and female mice is 100 ppm in the diet (males, 10.1 mg/kg/day; females, 10.3 mg/kg/day) based on effects on the kidney including tubular dilatation, chronic glomerulonephritis, and hyaline casts in female mice, and hyperplasia of the stomach mucosal epithelium in male mice. The NOAEL for male and female mice is 10 ppm in the diet (approximately 1 mg/kg/day).

Treatment of male and female ICR (Crj:CD-1) mice for up to 78 weeks with XRD-537 BE in the diet did not result in a statistically significant increase in the number of animals with primary neoplasms at any anatomical site. Common age related neoplasms included hepatocellular adenomas and lung adenomas/adenocarcinomas in male mice and lung adenomas/adenocarcinomas and malignant lymphomas in female mice. The doses used in this study were inadequate for assessing carcinogenicity.

C. STUDY DEFICIENCIES

Statistics should have been provided for main study animals (animals sacrificed at 78 weeks as well as animals in the main study found dead or sacrificed *in extremis*). The reviewer felt that total incidences, for which the study authors provided statistics, were not as important as main study incidences because some lesions did not appear until late in the study; animals sacrificed early, before lesions appeared, were effectively censored. The reviewer calculated probabilities for the main study animals and based LOAELs and NOAELs on main study animals.

DATA EVALUATION RECORD

CYHALOFOP BUTYL
(XRD-537 BE)

STUDY TYPE: CHRONIC TOXICITY/ONCOGENICITY ORAL STUDY - RAT
[OPPTS 870.4300 (§83-5)]
MRID 45000417

Prepared for

Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
1921 Jefferson Davis Highway
Arlington, VA 22202

Prepared by

Chemical Hazard Evaluation Group
Toxicology and Risk Analysis Section
Life Sciences Division
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Task Order No.01-81EE

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Disclaimer

This review may have been altered subsequent to the contractor's signatures above.

CYHALOFOP BUTYL, technical **Chronic Toxicity/Oncogenicity Oral Study [OPPTS 870.4300 (§ 83-5)]**

EPA Reviewer: John Whalan, Date 6-5-02
Registration Action Branch 2 (7509C)
EPA Work Assignment Manager: SanYvette Williams-Foy, D.V.M., Date 6/5/02
Registration Action Branch 2 (7509C)
TXR No.: 0050348

DATA EVALUATION RECORD

STUDY TYPE: Combined chronic toxicity/oncogenicity feeding- rat [OPPTS 870.4300 (§83-5)]

DP BARCODE: D268553
P.C. CODE: 082583

SUBMISSION CODE:
TOX. CHEM. NO.:

TEST MATERIAL (PURITY): XRD-537 BE (cyhalofop butyl) (97.1% a.i.)

SYNONYMS: R-(+)-n-Butyl-2-(4-(2-fluoro-4-cyanophenoxy)phenoxy)propanoate

CITATION: Harada, T. (1994) XRD-537 BE: 24-Month oral chronic toxicity and oncogenicity study in rats. Mitsukaido Laboratories, The Institute of Environmental Toxicology, 4321, Uchimoriya-cho, Mitsukaido-shi, Ibaraki 303, Japan. Laboratory Study ID: GHF-P-1387, June 2, 1994. MRID 45000417. Unpublished.

SPONSOR: Dow Chemical Japan, Ltd., DowElanco Division, Seavans North, 2-1, Shibaura 1-chome, Minato-ku, Tokyo 105, Japan.

Nichimen Corporation, 11-1, Nihonbashi 3-chome, Chuo-ku, Tokyo 103, Japan.

EXECUTIVE SUMMARY: In a chronic toxicity/oncogenicity study (MRID 45000417), XRD-537 BE (97.1%a.i.; Lot #: AGR 295713) was administered to 90 Fischer (F344/DuCrj) rats/sex/dose in diet at dose levels of 0, 3, 6, 24, or 100 ppm [0, 0.1020, 0.2047, 0.823, or 3.44 mg/kg/day] in males and 0, 6, 60, or 600 ppm (0, 0.2451, 2.475, or 24.97 mg/kg/day) in females for 104 weeks. Ten animals/sex/dose were killed at 13, 26, 52, or 78 weeks for interim evaluation.

The kidney was the target organ of XRD-537 BE in male and female rats. Histopathological examination revealed high-dose males and females had brown pigment deposition in the proximal tubular cells in the kidneys starting at week 52, while the first incidence of pigmentation in control and lower dose animals was not noted until 104 weeks. The incidence rates at 52 and 78 weeks were 7/10 and 8/10 for high-dose males, respectively, and 9/10 and 8/10 for high-dose females, respectively (vs. 0/10 controls; $p \leq 0.01$). At 104 weeks, 21/40 high-dose males and 33/38 high-dose females exhibited the increased pigment deposition, compared with 6/40 and 6/42 controls, respectively ($p \leq 0.01$). When considering all high-dose males and females, including those that died early or were killed *in extremis*, the incidence rates were 40/90 and 59/89 for males and females, respectively, compared with 8/90 and 6/90 controls, respectively ($p \leq 0.01$). In main study animals, the incidence rates were 25/50 and 42/50 for males and

females, respectively, compared with 8/50 and 6/50 controls, respectively ($p \leq 0.01$). The brown pigment was identified as lipofuscin and hemosiderin. Renal mineralization was also increased in main study high-dose females (22/50, $p<0.05$) compared with controls (13/50). No statistically increased incidence was observed in any of the other dose groups.

Treatment with XRD-537 BE did not adversely affect survival, clinical signs, body weight, food consumption, hematology, clinical chemistry, urinalysis, ophthalmologic findings, organ weights, or macroscopic findings. Other histopathological changes observed were not considered an adverse effect of treatment.

A LOAEL of 100 ppm (3.44 mg/kg/day) for male rats and of 600 ppm (24.97 mg/kg/day) for female rats was identified based on the early and increased deposition of the pigments lipofuscin and hemosiderin in the renal proximal tubular cells of both sexes and renal mineralization in female rats. The NOAEL was 24 ppm (0.823 mg/kg/day) for males and 60 ppm (2.475 mg/kg/day) for females.

At the doses tested, there was no treatment-related increase in tumor incidence when compared to controls. These doses were inadequate to assess the carcinogenic potential of cyhalofop butyl.

The chronic toxicity portion of this study is **Acceptable/Guideline** and does satisfy the guideline requirement for a chronic toxicity oral study in the rat. The carcinogenicity portion of the study is **Unacceptable/Guideline** because the doses tested were too low to elicit frank toxicity or to assess the carcinogenic potential of cyhalofop butyl in rats.

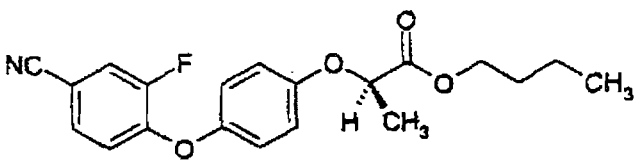
COMPLIANCE: Signed and dated GLP, Quality Assurance, Data Confidentiality, and Flagging statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test material: XRD-537 BE

Description: off-white powder
Lot/Batch No.: AGR 295713
Purity: 97.1% a.i.
Stability of compound: stable in a dark and cold environment
CAS No.: 122008-85-9
Structure:



2. Vehicle and/or positive control

The test material was administered in certified diet MF Mash. No positive control was used in this study.

3. Test animals

Species: Rat
Strain: Fischer (F344/DuCrj)
Age and weight at study initiation: approximately 5 weeks old; males: 93 - 123g; females; 72 - 93g
Source: Charles River Japan, Inc. (Atsugi Breeding Center: Shimofurusawa, Atsugi-shi, Kanagawa and Hino Breeding Center: Hino-cho, Gamoh-gun, Shiga)
Housing: Housed in groups of five (same sex) in aluminum cages with stainless steel wire-mesh floors (width 310 mm x depth 440 mm x height 230 mm)
Diet: Certified diet MF Mash (Oriental Yeast Co., Ltd., Azusawa, Itabashi-ku, Tokyo) was available *ad libitum*
Water: Well water (passed through precipitating and sedimentation procedures and sterilized with hypochlorous acid and ultraviolet light) was available *ad libitum*
Environmental conditions:
Temperature: 24±2°C
Humidity: 55±15%
Air changes: 15 times/hour
Photoperiod: 12 hours light/12 hours dark
Acclimation period: 10 days for males; 7 days for females

B. STUDY DESIGN

1. In life dates

Start: September 30, 1991; end: October 6, 1993

2. Animal assignment

Animals were randomly assigned to the test groups in Table 1 based on a computer-assisted randomization procedure based upon body weight.

TABLE 1: Study design								
Test Group	Concentration in Diet (ppm)		Dose to animal (mg/kg/day)		Main Study 24 months		Satellite Study* 13, 26, 52, 78 weeks	
	Male	Female	Male	Female	Male	Female	Male	Female
Control	0	0	0	0	50	50	40	40
Low (LDT)	3	6	0.1020	0.2451	50	50	40	40
Mid (MDT)	6	60	0.2047	2.475	50	50	40	40
High-mid	24	–	0.823	–	50	–	40	–
High (HDT)	100	600	3.44	24.97	50	50	40	40

* Ten males and 10 females were killed at each time point
Data taken from text tables on pp. 13 and 33; MRID 45000417.

3. Dose selection rationale

Dose selection was based on an oral subchronic toxicity study in rats (MRID 45014705). In that study, groups of twelve SPF Fisher (F344/DuCrj) rats of each sex were administered 0, 30, 300, 1000, or 3000 ppm XRD-537 BE in the diet for 13 weeks. No adverse effects were noted at 30 ppm in either sex, or in females at 300 ppm. The specific gravity of urine collected from 1000 or 3000 ppm males was elevated as compared with controls (15 and 20%, respectively). The study authors reported hematology changes indicative of anemia in males fed 1000 or 3000 ppm; however, the statistically significant changes were not biologically significant (i.e., changes were ≤6% of control values). Clinical chemistry analysis of males fed 300 ppm and greater revealed dose-related increases in blood urea nitrogen, albumin, and albumin/globulin ratio and dose-related decreases in globulin and total cholesterol. Males fed 1000 and 3000 ppm additionally had dose-related increases in alkaline phosphatase and decreased levels of glutamic oxaloacetic transaminase and glutamic pyruvic transaminase. Clinical chemistry analysis of blood from treated females did not reveal biologically significant changes. Autopsy revealed darkened and/or enlarged livers in 7/12 male rats fed 1000 ppm and all 3000 ppm males and 9/12 females fed 3000 ppm. Additionally, all male and female rats fed 3000 ppm had darkened kidneys. Dose-related increases in absolute and relative liver weights were observed in males fed 300 ppm or greater, and increased absolute and relative kidney weights and slightly decreased absolute and relative spleen weights were seen in males fed 1000 or 3000 ppm. Female rats fed 1000 or 3000 ppm showed dose-related increases in absolute and relative liver weights. Histopathological examination of the liver revealed hepatocellular swelling with minute cytoplasmic eosinophilic granules in 4/12 males fed 300 ppm and in all males and females fed 1000 or 3000 ppm. The kidneys in all males and females fed 3000 ppm had brown pigment deposition (lipofuscin) in the proximal tubular cells.

Based on the results from this study, the study authors concluded that the highest dose level in a 2 year chronic toxicity and oncogenicity study should be less than 300 ppm for males and less than 1000 ppm for females. Dose-levels higher than these might result in shortened life span due to kidney or liver toxicity. Therefore, the doses

selected for the chronic toxicity study were 0, 3, 6, 24, or 100 ppm for males and 0, 6, 60, or 600 ppm for females.

4. Diet preparation and analysis

Diet was prepared once prior to treatment initiation and then once every 4 weeks. Appropriate amounts of the test substance were first dissolved in acetone, and then mixed with part of the basal diet (certified diet MF Mash) to make a premixture. The premix was then blended with the remaining part of the diet by a mixer. After mixing, the diet was allowed to sit for 30 minutes to allow for evaporation of the acetone before being sealed in plastic bags. The diet was stored in the dark at 4°C. Homogeneity and stability were tested prior to the initiation of treatment on 50 g samples taken from the top, middle and bottom of the mixer for each dose level. Concentrations of the test substance in test diets at all dose levels were determined on samples from all lots of test diets prepared prior to initiation of treatment and during the treatment period. Stability and enantiomer ratio of the test substance in the test diet were determined on samples from the 600 ppm diet for females at the following time points: day 0, 42 days (at 4°C in dark room), 57 days (at 4°C in dark room for 42 days, followed by room temperature in the animal room for 15 days), and 64 days (at 4°C in dark room for 42 days, then room temperature in animal room for 15 days, followed by exposure to ambient air in a feeding jar for 7 days).

Results –

Homogeneity Analysis: The range of measured concentrations of the top, middle, and bottom samples for the 3, 6, 24, or 100 ppm male test diets were: 2.9-3.1, 5.7-5.8, 23-24, or 97-99 ppm, respectively, and for 6, 60 or 600 ppm female diets were: 5.9-6.1, 56-58, or 562-582 ppm, respectively. The coefficient of variation for each dose level for the male and female diets was ≤ 2.4%, indicating that the test substance was homogeneously mixed in the diets.

Stability Analysis: The results of the stability analysis were found only in the text. The study authors claim that the enantiomer ratio (R/S, %/%) in the diet was 99.0/1.0 or 98.9/1.1 at each time point, indicating that good stability was maintained during the study.

Concentration Analysis: The range of mean measured concentrations for the 3, 6, 24, or 100 ppm male test diets was: 2.8-3.2 ppm (93-107%), 5.4-6.5 ppm (90-108%), 22-24 (92-100%), and 92-98 (92-98%), respectively, and for the 6, 60, or 600 ppm female test diets were: 5.5-6.0 ppm (92-100%), 55-59 ppm (92-98%), and 549-582 (92-97%), respectively.

The analytical data indicated that the mixing procedure was adequate and that the variance between nominal and actual dosage to the animals was acceptable.

5. Statistics

Body weight, food consumption, urine volume and specific gravity, clinical chemistry parameters, and organ weights were analyzed using multiple comparison test (exact method not stated) followed by Dunnett's or Scheffe's test. The remaining urinalysis data were analyzed using the Mann-Whitney's U test. The life table analysis was used to assess mortality. Lastly, Fisher's exact probability test was used to analyze data pertaining to clinical signs, ophthalmology, and pathology results.

C. METHODS

1. Observations

Animals were inspected at least once daily for signs of toxicity and mortality.

2 Body weight

During treatment, animals were weighed weekly during the first 13 weeks, once at week 16, and once every 4 weeks thereafter.

3. Food consumption and compound intake

Food consumption for each animal was determined weekly during the first 13 weeks, once at week 16, and once every 4 weeks thereafter. Mean daily diet consumption was calculated as g food/rat/day. Food efficiency (body weight gain in g/food consumption in g per unit time × 100) for the first 13 weeks and compound intake (mg/kg/day) values were calculated as time-weighted averages from the consumption and body weight gain data.

4. Ophthalmoscopic examination

Eyes were examined in all animals (from the main groups and satellite groups) prior to the initiation of treatment and in all surviving animals in the highest dose-group and the control group from the main groups at 104 weeks of treatment.

5. Blood was collected for hematology and clinical chemistry analysis from 10 male and 10 female animals from each of the satellite groups at treatment weeks 13, 26, 52, and 78 weeks (only 9 females from each dose level at 78 weeks) and from all surviving animals at 104 weeks of treatment. Animals were fasted overnight prior to blood sampling. Blood samples were collected from the posterior vena cava following laparotomization under ether anesthesia. The CHECKED (X) parameters were examined.

a. Hematology

X		X	
X	Hematocrit (HCT)*	X	Leukocyte differential count*
X	Hemoglobin (HGB)*	X	Mean corpuscular HGB (MCH)
X	Leukocyte count (WBC)*	X	Mean corpuscular HGB concentration (MCHC)
X	Erythrocyte count (RBC)*	X	Mean corpuscular volume (MCV)
X	Platelet count*		Reticulocyte count
	Blood clotting measurements*		
	(Thromboplastin time)		
	(Thromboplastin time)		
	(Clotting time)		
	(Prothrombin time)		

* Required for chronic toxicity/oncogenicity based on Subdivision F Guidelines

b. Clinical chemistry

X	ELECTROLYTES	X	OTHER
X	Calcium*	X	Albumin*
X	Chloride*	X	Blood creatinine*
	Magnesium	X	Blood urea nitrogen*
X	Phosphorus*	X	Total Cholesterol
X	Potassium*	X	Globulins
X	Sodium*	X	Glucose*
		X	Total bilirubin
		X	Total serum protein*
	ENZYMES	X	Triglycerides
X	Alkaline phosphatase (ALK)		Serum protein electrophoresis
	Cholinesterase (ChE)	X	Albumin/globulin ratio
X	Creatine phosphokinase		
	Lactic acid dehydrogenase (LDH)		
X	Serum alanine aminotransferase* (SGPT)		
X	Serum aspartate amino-transferase* (SGOT)		
X	Gamma glutamyl transferase (GGT)		
	Glutamate dehydrogenase		

* Required for chronic toxicity/oncogenicity studies based on Subdivision F Guidelines

6. Urinalysis

Urine was collected from 10 male and 10 female animals from each of the satellite groups at treatment weeks 13, 26, 52, and 78 weeks (only 9 females from each dose level at 78 weeks) and from10 male and 10 female animals from each of the main groups at 104 weeks of treatment. The animals were not fasted prior to urinalysis. The CHECKED (X) parameters were examined.

X		X	
X	Appearance*	X	Glucose*
X	Volume*	X	Ketones*
X	Specific gravity*		Bilirubin
X	pH	X	Blood*
X	Sediment (microscopic)*		Nitrate
X	Protein*	X	Urobilinogen

*Required for chronic toxicity/oncogenicity studies based on Subdivision F Guidelines

7. Sacrifice and pathology

All animals that died and those sacrificed on schedule were subjected to gross pathological examination and the CHECKED (X) tissues were collected for microscopic examination. All tissues preserved from all animals (except those in the 13 week satellite group) were examined microscopically except the tongue, pharynx, larynx, and vagina. In addition, the [XX] organs were weighed.

X	DIGESTIVE SYSTEM	X	CARDIOVASC./HEMAT	X	NEUROLOGIC
X	Tongue	X	Aorta*	XX	Brain*+
X	Salivary glands*	XX	Heart*	X	Peripheral nerve*
X	Esophagus*	X	Bone marrow*	X	Spinal cord (3 levels)*
X	Stomach*	X	Lymph nodes*	X	Pituitary*
X	Duodenum*	XX	Spleen*	X	Eyes (optic nerve)*
X	Jejunum*	X	Thymus*		
X	Ileum*				GLANDULAR
X	Cecum*		UROGENITAL	XX	Adrenal gland*
X	Colon*	XX	Kidneys*+		Lacrimal gland
X	Rectum*	X	Urinary bladder*	X	Mammary gland*
XX	Liver*+	XX	Testes*+	X	Parathyroids*
X	Pancreas*	X	Epididymides	X	Thyroids*
		X	Prostate		
	RESPIRATORY	X	Seminal vesicle		OTHER
X	Trachea*	X	Ovaries*	X	Bone*
X	Lung*	X	Uterus*	X	Skeletal muscle*
X	Nose	X	Vagina	X	Skin*
X	Pharynx			X	All gross lesions and masses*
X	Larynx				

*Required for chronic toxicity/oncogenicity studies based on Subdivision F Guidelines.
+Organ weight required in chronic toxicity/oncogenicity studies.

II. RESULTS

A. OBSERVATIONS

1. Toxicity

No treatment-related clinical signs of toxicity were observed. Incidences of clinical signs in the treatment groups were comparable to controls.

2. Mortality

Treatment with XRD-537 BE did not cause an increase in mortality. The survival rates for the 0, 3, 6, 24, or 100 ppm males were 40/50 (80%), 42/50 (84%), 35/50 70%), 43/50 (86%), and 40/50 (80%), respectively, and for the 0, 6, 60, or 600 ppm females were 42/50 (84%), 45/50 (90%), 38/50 (76%), and 38/50 (76%), respectively.

B. BODY WEIGHT

Selected absolute body weights are presented in Table 2. No detrimental treatment-related effects on body weights were observed in treated males or females as compared with controls. Absolute body weights in 600 ppm females were statistically increased ($p<0.05$; 0.01) sporadically during the first 28 weeks of treatment and in the 60 ppm group at 4 weeks, but the weights were increased by only up to 4% of controls. The 24 ppm males had statistically increased ($p<0.05$) absolute body weight at week 104, but the increase was only 7% greater than controls. Body weight gain data were not provided.

TABLE 2. Selected mean body weights of main group male and female rats fed XRD-537 BE for up to 104 weeks

Weeks	Concentration (ppm)								
	0	3	6	24	100	0	6	60	600
	Males					Females			
	Mean body weights (kg) [a]								
0	108 ± 5	108 ± 5	108 ± 5	108 ± 5	108 ± 5	82 ± 4	82 ± 4	82 ± 4	82 ± 4
13	330 ± 16	330 ± 15	329 ± 17	333 ± 16	336 ± 19	194 ± 8	195 ± 9	196 ± 10	198 ± 10
52	455 ± 23	461 ± 22	454 ± 27	463 ± 26	466 ± 30	256 ± 15	257 ± 13	259 ± 15	262 ±19
76	489 ± 33	501 ± 24	483 ± 34	499 ± 30	501 ± 34	302 ± 29	305 ± 26	307 ± 30	311 ± 32
104	458 ± 55	486 ± 48	476 ± 49	492* ± 41	485 ± 37	332 ± 33	343 ± 33	333 ± 32	338 ± 37

Data taken from Tables 5-1 to 6-3, pp. 88-93; MRID 45000417.

Statistically different from controls: *$p \leq 0.05$.

C. FOOD CONSUMPTION AND COMPOUND INTAKE

1. Food consumption

No adverse, treatment-related effects on food consumption were observed. Food consumption was statistically increased ($p<0.05$; 0.01) occasionally in the 24 and 100 ppm males and 600 ppm females during the first 9 weeks of treatment, but they were increased by only up to 9%.

2. Compound consumption

Animals were given the test compound in the diet, and mean daily intake as a time-weighted average (mg compound/kg/day) for both sexes is given in Table 1.

3. Food efficiency

Food efficiency in treated male and female rats during the first 13 weeks of treatment was comparable to controls.

D. OPHTHALMOSCOPIC EXAMINATION

There were no treatment-related ophthalmologic findings in either sex.

E. BLOOD WORK

1. Hematology

No adverse, treatment-related changes in hematology were observed in satellite or main group males or females. The statistically significant values for RBC, mean corpuscular volume, and mean corpuscular hemoglobin observed in 24 and 100 ppm males at 13 weeks as compared with controls were not dose-related or biologically relevant. Statistically significant differences observed in hematocrit, hemoglobin, and/or RBC in the 6, 60, and/or 600 ppm females at weeks 13, 26, 104 were not related to dose. Females in the 600 ppm group had statistically ($p<0.05$; 0.01) increased WBC (+31%), lymphocytes (+26%), segmented neutrophils (+160%) at 26 weeks of treatment, and decreased platelets at 52 weeks (-14%).

2. Clinical chemistry

Statistically significant differences in clinical chemistry parameters in treated animals as compared to controls were transient and of questionable biological significance. In high-dose males, triglycerides were decreased at 13 weeks (-23% of controls; $p\leq0.01$) and 52 weeks (-13%; $p\leq0.05$); globulin levels were decreased at 52 weeks (-6%; $p\leq0.01$); the albumin:globulin ratio was increased at 13 weeks (+6%; $p\leq0.05$) and 52 weeks (+9%; $p\leq0.01$), and potassium levels were increased at 26 weeks (+8%). In high-dose females, total bilirubin was decreased at weeks 13 (-13%; $p\leq0.01$), 26 (-12%; $p\leq0.01$) and 52 (-11%; $p\leq0.05$); and triglycerides and cholesterol were

decreased at week 52 (-30% and -16%, respectively; p≤0.01 and 0.05; respectively). Other statistically significant differences noted were not related to dose.

F. URINALYSIS

No definitive, treatment-related effects were measured in the urinalysis. High-dose males had increased urine pH (2 animals had 8.0 pH vs. no animals in the control) and urine volume (+25%; p≤0.05) at 52 weeks compared with controls. High-dose females had elevated urine pH (1 animal had 8.5 pH vs. no animals in the control), and mid- and high-dose females had increased urine volume (+56% and +40%, respectively; p≤0.01) at 26 weeks of treatment. No other statistically significant changes in urinalysis were observed.

G. SACRIFICE AND PATHOLOGY

1. Organ weight

No consistent, biologically significant differences in organ weights were observed in treated males or females as compared with controls. High-dose males had statistically increased relative kidney weights compared with controls at weeks 26 and 78, but the increases were minimal (+9 and +8% of controls, respectively; p≤0.05; 0.01). Liver weights relative to body weights were statistically decreased at 52 weeks of treatment in males fed 6 or 100 ppm (-7% and -8% of controls, respectively; p≤0.05; 0.01). Relative testes weights were statistically increased in 24 ppm males at 52 weeks of treatment (+7%; p≤0.05). No other statistically significant differences were observed in treated males.

In high-dose females, relative liver weights were increased by 8% at 13 weeks of treatment (p≤0.01), while absolute and relative liver weights were increased at 26 weeks (+13% and +8%, respectively; p≤0.01). The only other statistically significant changes were increased absolute spleen weights (+9%; p≤0.01) and absolute kidney weights (+7%; p≤0.05) in high-dose females at 26 weeks of treatment as compared with controls.

2. Gross pathology

Selected macroscopic lesions are presented in Table 3. Possible treatment-related macroscopic lesions in males were limited to an increased incidence of dark-colored kidneys in males at 100 ppm. The first incidence was reported at 52 weeks (1/10 vs. 0/10 for controls), with the highest incidence reported at 78 weeks (6/10 vs. 0/10 for controls; p≤0.01). An increased incidence was not observed at 104 weeks in main group animals, however. When considering the total number of animals affected in the high-dose group, including those that died or were killed *in extremis*, the incidence was significantly increased as compared with controls (10/90 vs. 3/90 for controls; p≤0.05).

Possible treatment-related macroscopic changes in females were found only in the high-dose animals. The incidence of soiled fur in the external genitalia area was increased in main group, high-dose females at 104 weeks (8/38 vs. 1/42 for controls; $p \leq 0.01$), and in all high-dose females, including those that died or were killed *in extremis* (14/89 vs. 4/90; $p \leq 0.05$). High-dose females had a statistically increased incidence ($p \leq 0.01$) of dark-colored kidneys noted at the first interim period of 13 weeks and at every time period thereafter (i.e.,13, 26, 52, 78, and 104 weeks; incidences of 7/10, 10/10, 9/10, 8/9, and 25/38, respectively, vs. 0/10 for controls at 13, 26, 52, and 78 weeks and 0/42 for controls at 104 weeks).

Hepatic changes were also noted in high-dose females, but the changes appeared to be transitional or not clearly dose-related. A dark-colored liver was noted in 5/10 high-dose females at 13 weeks (vs. 0/10 for controls), while an enlarged liver was observed in 9/10 high-dose females at 26 weeks (0/10 for controls). Liver spots were observed at 104 weeks in 3/42, 7/45, 10/38 ($p<0.05$) and 10/38 ($p<0.05$) control, 6, 60, or 600 ppm main group females, respectively.

TABLE 3. Selected macroscopic changes in rats given dietary XRD-537 BE for 104 weeks

Parameter	Week	0 ppm	3 ppm	6 ppm	24 ppm	100 ppm
Males						
Kidneys: dark in color	13	– [a]	–	–	–	–
	26	–	–	–	–	–
	52	0/10	0/10	0/10	0/10	1/10
	78	0/10	0/10	0/10	0/10	6/10**
	104	1/40	1/42	1/35	0/43	2/40
	all [b]	3/90	1/90	1/90	0/90	10/90*
Females						
Parameter	**Week**	**0 ppm**	**6 ppm**	**60 ppm**	**600 ppm**	
External appearance: Soiled fur in external genitalia area	13	–	–	–	–	
	26	–	–	–	–	
	52	–	–	–	–	
	78	1/10	1/9	1/9	2/9	
	104	1/42	3/45	2/38	8/38**	
	all	4/90	4/89	9/89	14/89*	
Liver: dark in color	13	0/10	0/10	0/10	5/10*	
	26	–	–	–	–	
	52	–	–	–	–	
	78	–	–	–	–	
	104	–	–	–	–	
	all	0/90	0/89	1/89	5/89*	
Liver: spots	13	–	–	–	–	
	26	–	–	–	–	
	52	–	–	–	–	
	78	0/10	1/9	0/9	0/9	
	104	3/42	7/45	10/38*	10/38*	
	all	3/90	8/89	11/89*	10/89*	
Liver: enlarged	13	–	–	–	–	
	26	0/10	0/10	0/10	9/10**	
	52	–	–	–	–	
	78	–	–	–	–	
	104	–	–	– q	–	
	all	1/90	0/89	1/89	10/89**	
Kidney: dark in color	13	0/10	0/10	0/10	7/10**	
	26	0/10	0/10	0/10	10/10**	
	52	0/10	0/10	0/10	9/10**	
	78	0/10	1/9	0/9	8/9**	
	104	0/42	0/45	0/38	25/38**	
	all	0/90	1/89	2/89	62/89**	

Data taken from Tables 21-1 to 22-11, pp. 168-191; MRID 45000417.

[a] No incidences reported in the 10 animals examined from each group at the selected time point.

[b] All animals including those that died early or were killed *in extremis*.

Statistically significant as compared with controls: *p≤0.05; **p≤0.01.

3. Microscopic pathology

a) Non-neoplastic – A summary of selected non-neoplastic lesions is presented in Table 4. High-dose males and females had increased brown pigment deposition in the proximal tubular cells in the kidneys starting at week 52. At 104 weeks, 21/40 high-dose males and 33/38 high-dose females exhibited the increased pigment deposition ($p \leq 0.01$), compared with 6/40 and 6/42 controls, respectively. Additionally, the incidence of brown pigment in the kidneys in animals killed *in extremis* was 4/10 for high-dose males and 9/12 for high-dose females, compared with 2/10 and 0/8 controls, respectively. Totals for males in the main study, calculated by the reviewer, were 8/50, 7/50, 6/50, 6/50, and 25/50 ($p<0.01$). Totals for females in the main study, calculated by the reviewer, were 6/50, 7/50, 7/50, and 42/50 ($p<0.01$). The brown pigment was identified as lipofuscin and hemosiderin. High-dose females had an increased incidence of mineralization in the kidney when considering all high-dose females in the study including those that died early or were killed *in extremis*. Incidences in main study females in ascending dose order were: 13/50, 16/50, 18/50, and 22/50 ($p<0.05$). This lesion was generally not observed in male rats at any sacrifice time. High-dose females also had increased incidence of hepatocellular swelling with minute eosinophilic granules in the liver starting at week 26. The incidence rates were 10/10, 10/10, 8/10, and 30/38 at 26, 52, 78, and 104 weeks, respectively, vs. no affected controls at any of the time points.

Other statistically significant differences were clearly not related to dose or were observed at only one interim time point.

TABLE 4. Selected non-neoplastic changes in rats given dietary XRD-537 BE for 104 weeks

Parameter	Week	0 ppm	3 ppm	6 ppm	24 ppm	100 ppm
Males						
Kidney: increased brown pigment deposition in proximal tubular cells	13	– [a]	–	–	–	–
	26	–	–	–	–	–
	52	0/10	0/10	0/10	0/10	7/10**
	78	0/10	0/10	0/10	0/10	8/10**
	104	6/40	6/42	3/35	5/43	21/40**
	all [b]	8/90	7/90	6/90	6/90	40/90**

Parameter	Week	0 ppm	6 ppm	60 ppm	600 ppm
Females					
Kidney: increased brown pigment deposition in proximal tubular cells	13	–	–	–	–
	26	–	–	–	–
	52	0/10	0/10	0/10	9/10**
	78	0/10	0/10	0/10	8/10**
	104	6/42	5/45	5/38	33/38**
	all	6/90	7/89	7/89	59/89**
Kidney: Mineralization	13	–	–	–	–
	26	5/10	6/10	4/10	7/10
	52	6/10	4/10	6/10	6/10
	78	1/10	3/10	3/10	3/10
	104	9/42	14/45	13/38	15/38
	all	26/90	29/89	31/89	38/89*
Liver: Hepatocellular swelling with minute eosinophilic granules	13	–	–	–	–
	26	0/10	0/10	0/10	10/10**
	52	0/10	0/10	0/10	10/10**
	78	0/10	0/10	0/10	8/10**
	104	0/42	0/45	0/38	30/38**
	all	0/90	0/89	0/89	63/89**

Data taken from Tables 27-1 to 28-14, pp. 274-304; MRID 45000417.

[a] No incidences reported in the 10 animals examined from each group at the selected time point.

[b] All animals including those that died early or were killed *in extremis*.

Statistically significant as compared with controls: *p≤0.05; **p≤0.01.

b) Neoplastic – No treatment-related neoplastic lesions were observed in treated animals as compared with controls. Statistically significant differences were limited to an increased (p≤0.05) incidence of interstitial cell tumors in the testis of males in the 6 and 100 ppm groups that were killed *in extremis* (incidence rates for the 0, 3, 6, 24, or 100 ppm groups were 4/10, 5/8, 13/15, 5/7, and 9/10, respectively). When considering males from the satellite groups or the main group, no statistically significant increases were observed [incidences in the main group ranged from 42/50 (controls) to 47/50 (100 ppm)]. The only other statistically significant difference observed was a decreased incidence of adrenal pheochromocytoma in 3 ppm males at 104 weeks of treatment. The most common tumor type in male rats, including controls, was testicular interstitial cell tumors (present in 84-94% of males). No statistically significant differences in

neoplastic lesions were observed in any of the exposed female groups as compared with controls. The most common tumor types in all groups of female rats, including controls, were endometrial stromal polyps (14-19% of females) and pituitary adenomas (16-24% of females).

III. DISCUSSION

A. INVESTIGATOR'S CONCLUSIONS

The investigators concluded that the target organs are the liver and kidney. The minimum and sure toxic level in males was 100 ppm based on urinalysis results (higher pH and urine volume at 52 weeks), blood biochemistry changes (lower globulin and triglyceride levels, and higher albumin/globulin ratio at 13, 26, and/or 52 weeks of treatment), increased incidence of dark-colored kidney after week 78, increased relative kidney weights after 26 and 78 weeks of treatment, and increased incidence of brown pigment deposition in the renal proximal tubular cells after 52, 78, and 104 weeks of treatment. The corresponding no-effect level for males was 24 ppm.

In females, the investigators concluded that the sure toxic level in females was 600 ppm based on urinalysis results (higher pH and urine volume at week 26; blood biochemistry changes (lower total cholesterol and triglyceride levels at 52 weeks); increased incidence of dark-colored and enlarged livers at 13 and 26 weeks of treatment, respectively; increased absolute and/or relative liver and kidney weights at 13 and/or 26 weeks of treatment; increased incidence of hepatocellular swelling with minute eosinophilic granules after 26 weeks of treatment; and increased incidence of brown pigment deposition in the renal proximal tubules after 52 weeks of treatment. The minimum toxic level in females was 60 ppm based on higher urine volume at week 26. The no-effect level was 6 ppm.

B. REVIEWER'S DISCUSSION/CONCLUSIONS

Treatment with XRD-537 BE at dietary levels of up to 100 ppm in males and 600 ppm in females for up to 104 weeks did not adversely affect clinical signs, survival rates, mean body weights, food consumption, food efficiency, or ophthalmologic findings. Statistically significant hematology changes were not considered treatment-related changes because the changes were not dose-related and/or were not biologically relevant. The increased incidence of soiled fur in the external genital area observed during macroscopic examination of main group, high-dose females was not considered an adverse effect of treatment because it did not consistently correlate with any digestive or renal changes.

The kidney appeared to be the target organ of toxicity for XRD-537 BE. Histopathologic examination of the kidneys revealed increased brown pigment deposition in the proximal tubular cells in high-dose males and females starting at week 52. Staining of the pigment revealed that it was comprised of lipofuscin and hemosiderin. The early and increased incidence of the pigmentation was considered to be an adverse effect of treatment.

Other changes in the kidney, however, were either not clearly related to treatment or did not represent an adverse effect. The reviewer disagrees with the study author that the changes observed in urinalysis should be considered an adverse effect. The changes observed were transient in nature and were limited to increased pH and urine volume at only 26 weeks (females) or 52 weeks of treatment (males). The increases in kidney weights cited by the study author were transient and not biologically significant (increases of <10% in relative kidney weights in high-dose males at 26 and 78 weeks and in absolute kidney weights in high-dose females at 26 weeks).

Macroscopic evaluation revealed an increased incidence of dark-colored kidneys in high-dose males and females. At first glance, one would assume the dark coloration of the kidneys would coincide with the presence of the pigment in the kidney. However, the incidence of dark-colored kidneys in high-dose males was increased only at 78 weeks and when considering all high-dose males from all time points, including those that died early or where killed *in extremis*. In high-dose females, the incidence of dark colored kidneys was statistically increased at all time points starting as early as 13 weeks. The increased incidence of pigment deposition in the proximal tubular cells, however, was not observed until week 52. Therefore, it is unclear what was responsible for the dark coloration of the kidneys at the interim sacrifices. Because the dark-coloration does not consistently correlate with any other indices of renal toxicity, it is not by itself considered an adverse effect of treatment. Another renal effect noted in high-dose females was mineralization in the kidneys. Although incidences of renal mineralization were similar among females early in the study and incidences in the 600 ppm main study group were only slightly elevated over controls (13/50 vs 22/50), treatment appeared to accelerate this lesion in aging females. Therefore, kidney mineralization was considered a minimal, but adverse, treatment-related effect.

The treatment-related changes associated with the liver were not considered adverse by the reviewer. In high-dose males, the changes in the globulin levels and albumin/globulin ratio were transient and not biologically significant. The decreases in triglycerides in high-dose males were also transient (occurring at 13 weeks and 52 weeks) and were not accompanied by other indices of liver toxicity (i.e., changes in liver weights or pathology). In high-dose females, hepatic changes were generally transient in nature and did not consistently indicate hepatotoxicity. Clinical chemistry changes were limited to decreased bilirubin levels at weeks 13, 26, and 52 and decreased triglyceride and cholesterol levels at week 52. The changes in bilirubin levels were considered biologically insignificant. Changes in liver weights were limited to biologically insignificant increases in relative liver weights at 13 weeks (only 8%) and absolute and relative liver weights at 26 weeks (13 and 8%, respectively). Gross hepatic changes in high-dose females were limited to a dark liver observed only at 13 weeks and an enlarged liver observed only at 26 weeks. Liver spots were statistically increased at 104 weeks of treatment, but the number of animals affected in the 60 or 600 ppm groups were the same and therefore did not demonstrate an effect of dose. From these data, no clear indication of hepatotoxicity is provided. The histopathological lesion of hepatocellular swelling with minute eosinophilic granules was observed in almost all high-dose females starting at 26 weeks of treatment. This lesion was considered by the reviewer to simply reflect an adaptive response to exposure to a xenobiotic and does not represent an adverse effect.

A LOAEL of 100 ppm (3.44 mg/kg/day) for male rats and of 600 ppm (24.97 mg/kg/day) for female rats was identified based on the early and increased deposition of the pigments lipofuscin and hemosiderin in the renal proximal tubular cells of both sexes and mineralization of the kidneys of female rats. The NOAEL was 24 ppm (0.823 mg/kg/day) for males and 60 ppm (2.475 mg/kg/day) for females.

Treatment of Fischer 344 rats with XRD-537 BE for 104 weeks did not result in statistically significant treatment-related increases in tumor incidences under the conditions of this study. The doses tested were too low to elicit frank toxicity and inadequate to assess the carcinogenic potential of cyhalofop butyl in rats.

C. STUDY DEFICIENCIES

The reviewer was not able to make an accurate assessment of the stability data of the test compound in the diet. Raw data showing the results of the stability testing were not provided for each time point of interest. Additionally, information about the breakdown products was not included, so the reviewer must take the word of the study authors that the enantiomer ratio obtained for each of the time points of interest provides an accurate indication as to the stability of the compound. These data should be available from at least one of the other studies submitted. A minor deficiency is that body weight gain data were not provided. However, a view of the absolute body weight data demonstrate that no adverse effects on body weight were present.

Statistical data were not presented for main study animals alone.

CYHALOFOP-BUTYL BACTERIAL/MAMMALIAN ACTIVATION; REVERSE GENE MUTATION (84-2)

EPA Reviewer: Irving Mauer, Ph.D. Date: 11/06/00
Reregistration Action Branch 3, HED (7509C)
EPA Secondary Reviewer: Nancy McCarroll Date: 10/05/00
Toxicology Branch 1, HED (7509C)
TXR No: 0050348 DP D268553 04/05/2001

DATA EVALUATION RECORD

STUDY TYPE: *Salmonella typhimurium* and *Escherichia coli*/mammalian activation gene mutation assay; OPPTS 870.5100 [§84-2]

DP BARCODE: D268553 (sub-bean to D267558) SUBMISSION CODE: S582655

P.C. CODE: 082583 TOX. CHEM. NO.: None

TEST MATERIAL (PURITY): XDE-537 (cyhalofop-butyl, 97.4% a.i.)

SYNONYMS: R-(+)-n-butyl 2-[4-(2-fluoro-4-cyanophenoxy) phenoxy] propionate.

CITATION: Watanabe, K. and Kato, T. (1991). XDE-537 BE Reverse Mutation Test, (IET 90-0177), performed at Kodaira Laboratories, The Institute of Environmental Toxicology, Kodaira, Tokyo (Japan), Laboratory Study No. GHF-R 257, dated 15 July 1991. MRID 45000421. Unpublished.

SPONSOR: Dow Elanco Japan Ltd., for Dow AgroSciences LLC, Midland, MI

EXECUTIVE SUMMARY: In independent reverse gene mutation assays in bacteria (MRID 45000421) four histidine auxotroph (*his*) strains of *Salmonella typhimurium* (TA100, TA 1535, TA98 and TA1537) and *Escherichia coli* tryptophane auxotrophic strain WP2 uvrA were exposed to 5 dose levels per plate (313, 625, 1250, 2500 and 5000 μg/plate) of XDE-537 (cyhalofop-butyl 97.4%.a.i. in DMSO) at 37° C in the presence and absence of mammalian metabolic activation (± S9 mix) , and the number of revertant colonies was counted after 48 hours of treatment. In addition to (DMSO) vehicle controls, cultures were exposed to strain specific and activation-prone mutagens, to serve as positive controls. Two independent assays were conducted in each bacterial tester strain.

XDE-537 was tested up to 5000 μg/plate ± S9; precipitation was found at 2500 and 5000 μg/plate in the absence of metabolic activation, but not in its presence. Increases in mean number of revertant colonies in any strain at any dose with/without activation were comparable to vehicle controls (*i.e.*, none greater than 2-fold). The positive controls induced the expected mutagenic responses in the appropriate strain. **Hence, it is concluded that XDE-537 (cyhalofop-butyl) is non-mutagenic for *Salmonella* TA strains and *E. coli* WP2 uvrA.**

This study is classified as ACCEPTABLE, and satisfies the requirement for FIFRA Test Guideline 84-2 for *in vitro* mutagenicity (bacterial reverse gene mutation) data.

COMPLIANCE: Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided.

I. MATERIALS AND METHODS

A. Materials

1. Test Material: XDE-537 (technical cyhalofop-butyl)
Description: Off white powder
Lot/Batch No.: AGR 284267
Purity: 97.4% a.i.
Stability of compound: "Stable in dark cold room: (where also stored)".
Solubility: Water, < 1.0 ppm
Solvent used: Dimethylsulfoxide (DMSO)

2. Control Materials:
Negative: None
Solvent/final concentration: DMSO, 5%
Positive: Non-activated cultures:

AF-2[1]	for TA100, TA98 and WP2 *uvrA*
Sodium azide	for TA1535
9-Aminoacridine	for TA1537

Activated cultures:

2-Aminoanthracene (2-anthramine) for all strains.

3. Activation: S9 derived from male Sprague-Dawley rats:

x	Aroclor 1254	x	induced	x	rat	x	liver

The activity of this S9 fraction was checked by measuring nutagenicity of 7, 12-dimethylbenz(a)anthracene (DMBA) and 2-aminoanthracene (2AA) in *S. typhimurium* TA100 and T98.

[1]2-(2-furyl)-3-(5 nitro-2-furyl acylamide)

S9 mix:

$MgCl_2$	8 mM
KCl	33 mM
G-6-PO_4	5 mM
NADPH	4 mM
NADH	4 mM
Sodium PO_4 buffer	100 mM (pH, 7.4)
S9 fraction	0.1 mL

4. Test Organisms: *S. typhimurium* strains

___TA97 __x__TA98 __x__TA100 ____TA102 ____TA104
__x__TA1535 __x__TA1537 ____TA1538

Other strain(s): *E. coli* WP2 uvrA

Properly maintained? Yes

Checked for appropriate genetic markers? Yes

5. Test compound Concentrations Used:

Cytotoxicity test : 200 to 5000 μg/plate
Main assay:
Nonactivated conditions: 313, 625, 1250, 2500, 5000 μg/plate
Activated conditions: 313, 625, 1250, 2500, 5000 μg/plate

B. Test Performance: XDE-537 was dissolved in DMSO at a concentration of 50 mg/mL, and 5 doses ranging from 313 to 5000 μg/plate applied to bacterial cultures. Toxicity was judged by a reduction in number of revertants and/or clearing of the background lawn.

1. Type of *Salmonella* assay:

__x__ standard plate test
____ preinduction (____ minutes)
____ "prival" modification
____ spot test
____ other (describe)

2. Protocol: An aliquot of 2 mL of molten soft agar containing either 0.5 mM histidine plus 0-5 mM D-biotin (for *Salmonella* strains), or 0-5 mM L-tryptophane (for *E. coli* WP-2), plus 0.1 mL of test substance, and either 100 mM sodium phosphate buffer or S9 mix added. Contents were mixed uniformly and overlaid on minimal glucose agar plates (30 mL/Petri dish), which contained Vogel-Bonner E medium supplemented with 1.5% agar and 2% glucose. Triplicate plates per dose were inoculated at 37°C for 48 hours, following which the number of test revertant colonies were counted, and compared to vehicle (DMSO) control values.

3. Evaluation (Criteria of Results): Results were judged positive without statistical analyses (based on replicate experiments) when (i) there was a twofold or greater increase above solvent (DMSO) in mean number of revertants; (ii) the increase in number of revertants was accompanied by a dose-response relationship; and (iii) the increase in revertants was reproducible.

II REPORTED RESULTS

A. Preliminary cytotoxicity assay: XDE-537 produced no cytotoxicity up to the HDT, 5000 μg/plate (Table 1, MRID 45000421, page 9)

B. Mutagenicity assay: In two independent (repeat) experiments, the average number of revertant colonies was comparable to vehicle (DMSO) control values up to the HDT, 5000 μg/plate, ± S9 [Tables 2 (1) to 3 (2), MRID 45000421, pages 10-13, attached]. In contrast, all mutagenic chemicals (positive controls) showed significantly increased frequencies of revertants.

The test article formed precipitation at the higher dose levels (2500 and 5000 μg/plate) in the absence of S9 mix, but not in its presence.

Therefore XDE-537 (cyhalofop-butyl technical) is not mutagenic to *Salmonella*

TA strains and *Escherichia coli* WP2 *uvrA* at conditions under which this study was conducted.

III REVIEWER'S DISCUSSION/CONCLUSIONS

A. The reviewer agrees with the investigators' conclusions based upon the well recognized procedures under which this type of study was conducted.

B. Study deficiencies: None

CYHALOFOP-BUTYL MAMMALIAN CELLS IN CULTURE; GENE MUTATION (84-2)

EPA Reviewer: Irving Mauer, Ph.D. Date: 11/06/00
Registration Action Branch 3, HED (7509C)
EPA Secondary Reviewer: Date: 12/04/00
Toxicology Branch I, HED (7509C)
TXR No: 0050348

STUDY TYPE: Mammalian cells in culture gene mutation assay in mouse lymphoma L5178Y cells; OPPTS 870.5300 (84-2)

DP BARCODE: D268553 (subbean to D267558) SUBMISSION CODE: S582655

P. C. CODE: 082583 TOX. CHEM. NO. None

TEST MATERIAL (PURITY): DE-537 N-butyl ester (cyhalofop-butyl, 97.4% a.i.)

SYNONYMS: Butyl-R-2-4-(4-cyano-2-fluorophenoxy-propionate)

CITATION: Adams, K. and Ransome, S. (1996). DE-537 N-Butyl Ester - Mammalian Cell Mutation Assay, performed at Huntingdon Life Sciences, Ltd., Cambridgeshire, England, Laboratory Project DWC 740/962438, 3 October 1996. MRID 45014711. Unpublished.

SPONSOR: Dow AgroSciences LLC, Midland MI, agent for Dow Elanco Europe, Wantage, Oxon (UK)

EXECUTIVE SUMMARY: In independent mammalian cell forward gene mutation assays (MRID 45014711), heterozygous thymidine kinase cells (TK +/-) of the mouse lymphoma L5178Y cell line were exposed to DE-537 (cyhalofop-butyl, 97.4% a.i. in dimethylsulfoxide) at concentrations ranging from 25 to 400 μg/mL for 3 hours in the presence and absence of metabolic activation (rat S9 Mix), and conversion to thymidine deficiency genotype (TK-/-) determined after a 48-hour expression period, followed by incubation for 11 to 12 days in selective medium.. In addition to solvent (DMSO) controls, cultures were treated with the mutagens, methyl methane sulfonate (MMS), and 20 methylcholanthrene, to serve as positive controls for the non-activation and activation test series, respectively.

DE-537 was tested up to cytotoxic (20 to 50% of solvent control) and precipitating levels (200 μg/mL and above). The positive controls induced the expected mutagenic responses. Although some individual significant increases in the mutant frequencies (outside the current historical range) were observed in the initial nonactivated tests, they were not above the concurrent control levels nor were they dose-related, or confirmed in the repeat tests. Colony-size distribution was unaffected by DE-537-n-butyl ester treatment. **It is concluded that DE-237-n-butyl ester (cyhalofop-butyl) was nonmutagenic in this *in vitro* mammalian cell mutation assay.**

This study is classified as ACCEPTABLE and satisfies the FIFRA Test Guideline for *in vitro* mammalian cell mutation data.

COMPLIANCE: Signed and dated GLP, Quality Assurance, and Data Confidentiality Statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material: DE-537 n-butyl ester
Description: Off-white powder
Lot/batch No.: AGR 295713
Purity: 97.4% a.i.
Stability of compound: Not reported.
Storage: "Room temperature":
Analysis of achieved concentration: Not performed.
Solvent used: Dimethylsulfoxide (DMSO)

2. Control Materials:
Negative: None
Solvent/final concentration: DMSO, 1%
Positive: Nonactivation (concentrations, solvent): methyl methane sulphonate (MMS), 10 μg/mL in DMSO
Activation (concentrations, solvent): 20-methylcholanthrene (MC), 2.5 μg/mL, in DMSO

3. Activation: S9 derived from male Sprague-Dawley rats (from Harlan Olac Ltd):

x	Aroclor 1254	x	induced	x	rat	x	liver
	phenobarbital		non-induced		mouse		lung
	none				hamster		other
	other				other		

S9 Mix Composition:

Isocitric acid .... 8.7 mM

NADP ..... 1.6 mM

S9 fraction ..... 5% v/v in ice-cold RoP (see below)

4. Test Cells: mammalian cells in culture:
__x__ Mouse lymphoma L5178Y cells (3.7.2C sub-strain)
_____ Chinese hamster ovary (CHO) cells
_____ V79 cells (Chinese hamster lung fibroblasts)
_____ Other (list):

Properly maintained? Yes
Periodically checked for Mycoplasma contamination? No
Periodically checked for karyotype stability? No
Periodically "cleansed" against high spontaneous background? Yes

5. Media:

RPMI 1640 (obtained from Imperial Laboratories) supplemented with 0.1% Synperonic (Serva), 0.011% sodium pyruvate (Sigma), 2mM L-glutamine (Sigma), 50 μg/mL gentamycin (Biol. Indus.) and buffered with 2 mg/mL sodium bicarbonate; the mixture referred to as "RoP"

6. Locus Examined:

__x__ Thymidine kinase (TK)
Selection agent: _____ Bromodeoxyurdidine (BrdU)
(Give concentration) _____ Fluorodeoxyuridine (FdU)
4 μg/mL Trifluorothymidine (TFT)

_____ Hypoxanthine-guanine-phosphoribosyl transferase (HPRT)
Selection agent: _____ 8-azaguanine (8-AG)
(Give concentration) _____ 6-thioguanine (6-TG)

_____ Na^+/K^+ ATPase
Section agent: _____ Ouabain
(Give concentration)

_____ Other (locus and/or selection agent; give details):

7. Test compound concentrations used:

a. Preliminary toxicity test: 1, 2.5, 5, 10, 25, 50, 100, 200, 300 μg/mL; harvested after 24 and 48 hours

b. Mutagenicity tests: Non-activated/activated conditions (2 tests): 25, 50, 100, 150, 200, 250, 300, 400 μg/mL.

B. TEST PERFORMANCE

1. Cell treatment:

a. Cells exposed to test compound, negative/solvent or positive controls for:

__3__ hours (non-activated) __3__ hours (activated)

b. After washing, cells cultured for __2__ days (expression period before cell selection:

c. After expression, 2 x 10^5 cells/dish (__3__ dishes/group) were cultured for __2__ days in selection medium to determine numbers of mutants and __200__ cells/dish (__3__ dishes/group) were cultured for 11 - 12 days without selective agent to determine cloning efficiennncy.

2. Evaluation of Results: Growth in suspension was calculated s follows:

$$\frac{\text{Cell count 24 hours post-treatment}}{2 \times 10^5} \times \frac{\text{Cell count 48 hours post-treatment}}{2 \times 10^5}$$

Relative Total Growth (RTG) was estimated by:

$$\text{RTG} = \frac{\text{Suspension growth (\% control)} \times \text{Viability in gar (\% control)}}{100}$$

Mutant Frequency (MF) per 10^6 survivors ws calculated as follows:

$$\frac{600}{\text{Total number of viable colonies}} \times \text{Total number of mutant colonies}$$

Statistical significance was assessed by weighted ANOVAs reported by Arlett *et al.* (1989)

3. Evaluation Methods: Criteria for a positive response were an increase in MF of the treated cultures by at least 100 relative to concurrent controls, with an associated increase in absolute colony numbers; statistically increase in MF following treatment with test chemical; statistically significant dose-relationship in any increased MF; observed increases in MF over historical and concurrent controls with RTG no less than 10 to 20%; reproducible and statistically significant increased MF.

II. REPORTED RESULTS

A. Preliminary Cytotoxicitty Assay: Relative growth in suspension following treatment with 1 to 300 μg/mL ranged from 107 down to 27% (-S9) and 214 to 50% (+S9) (Tables 1 and 2, MRID 45014711, pages 26 and 27). Compound precipitation was also recorded at ≥ 200 μ/mL +/-S9. Based on these data, eight doses (25 - 400 μg/mL) were selected for these nonactivted and S9-activated mutation assay.

B. Mutagenicity Assays: Precipitation was observed at the 5 highest concentrations tested (150, 200, 250, 300 and 400 μg/mL) and maximum cytotoxicity was seen at these precipitating dose levels. In nonactivated test cultures, both tests at 25 to 100 μg/mL resulted in mean relative cell growths of 71 to 30% and 92 to 49%, respectively. Cloned cultures treated at 50, 100, 200 and 400 μg/mL in both tests resulted in total growth levels of 62 at 50 μg/mL down to 24% at 400 μg/mL in the first, and 69 to 38% in the second assay.

Although sporadic statistically significant increases in MF outside the historical control range were recorded in the first non-activated tests, these increases were not 100 above control level, dose-related, nor reproduced in the second test (Table 2 to 5, MRID 45014711, pages 28 to 31, attached). Therefore, the investigators considered these increases to be not of biological significance. The positive control culture (MMS-treated) induced a highly significant ($p < 0.001$) increase in the MF.

In S9-activated tests, cultures treated with 25 to 400 μg/mL showed no consistent significant increases in MF in the two tests, (Tables 6 to 9, MRID 45014711, pages 32 to 35, attached). The positive control (MC) on the other hand, demonstrated the induction of highly significant ($p < 0.001$) increases in the MF in both trials.

Hence, the investigators concluded that DE-537 n-butyl ester was non-mutagenic

in this mammalian cell mutation test assay.

Determination of colony size distribution appeared to be unaffected by the test compound. (Table 10, 11; MRID 45014711, pages 36, 37, attached).

Current historical control data, updated to August 1996, was provided as follows:

	Lowest Level Recorded	Highest Level Recorded	Mean	SD
Solvent control (DMSO)	73	210	110	41.25
Positive control (MMS or MC)	290	1582	687	225.00

III. REVIEWER'S DISCUSSION/CONCLUSIONS:

The EPA reviewer agrees with the conclusions arrived at by the investigators utilizing recognized procedures for this assay, *i.e.*, that this chemical does not demonstrate mutagenic potential in L5178Y mouse lymphoma cells, treated up to precipitating and cytotoxic levels.

CYHALOFOP-BUTYL *IN VITRO* CHROMOSOME ABERRATIONS (84-2)

EPA Reviewer: Irving Mauer, Ph.D. Date: 11/06/00
Registration Action Branch 3, HED (7509C)
EPA Secondary Reviewer: Nancy McCarroll Date: 12/04/00
Toxicology Branch 1, HED (7509C)
TXR No. 0050348 01/05/2001

DATA EVALUATION RECORD

STUDY TYPE: *In vitro* mammalian chromosome aberrations; OPPTS, 870.5375 [§ 84-2] in Chinese hamster lung (CHL) cells

DP BARCODE: D268553 (subbean to D267558) SUBMISSION CODE: S582655

PC CODE: 082583 TOX. CHEM. NO.: None

TEST MATERIAL (PURITY): XDE-537 (cyhalofop, 97.4% a.i.)

SYNONYMS: R-(+)-n-butyl 2-[4-(2-fluoro-4-cyanophenoxy) phenoxy] propionate; XRD-537

CITATION: Matsumura, H., Matsumoto, K., Kato, T., and Ohta, T., (1991). XDE-537 *in vitro* Cytogenetics Test (IET 90-0178), performed at Kodaira Laboratories, Institute of Environmental Toxicology, Tokyo (Japan), Laboratory Study ID No. GHF-R 273, dated 9 May 1991. MRID 45000423. Unpublished.

SPONSOR: Dow Elanco Japan Ltd., Tokyo, Japan, for Dow AgroSciences, Midland, MI

EXECUTIVE SUMMARY:

In a mammalian cell cytogenetics assay for chromosome aberrations (MRID 45000423), Chinese hamster lung (CHL or V79) cell cultures were exposed to 312-5000 μg/mL cyhalofop-butyl as XDE-537 (97.4% a.i. in dimethylsulfoxide, DMSO) for 24 or 48 hours in the absence of mammalian metabolic activation, and for 6 hours in the presence of a mammalian metabolic activation system consisting of rat liver microsomes (S9 Mix), followed by 18 hours in fresh tissue culture medium. At cell harvest, cultures were scored for both structural and numerical aberrations. In addition to untreated and vehicle (DMSO) controls, cultures were treated with the clastogens mitomycin-C (MMC), and benzo(a)pyrene (BaP), to serve as positive controls for the non-activated and activated series, respectively.

Cyhalofop-butyl was tested up to slightly precipitating concentrations, 312 and 625 *ug*/mL (but the chemical dissolved completely at 156 *ug*/mL and below). In the tests without S9, the chemical dissolved almost completely during the 24-hour and 48-hour incubations; however, at concentrations of 1250 *ug*/mL and above, heavy precipitation was observed forming a thin layer

floating on the surface of the culture medium. The frequencies of structural chromosome aberrations in test cultures were comparable to both the concurrent vehicle (DMSO) and historical control values, *i.e.*, less than 5% (1.5 to 4%) after both 24 and 48-hour treatments. On the other hand, while the frequencies of polyploid metaphases were less than 5 % after the 24-hour treatment with the chemical, those after 48-hour treatment were 17.0 nd 16.5% at 312 and 625 *u*g/mL, respectively. These increased values were confirmed in a reexamination at 7 dose levels from 39.1 to 2500 *u*g/mL cyhalofop-butyl; increased values of polyploidy were comparable at 312 and 625 μg/mL, but gradually reduced above 625 *u*g/mL exposure (considered by the investigator due to insufficient interaction between cells on the surface of dish and precipitated test substance floating on the surface of the culture medium).

Among S9 activated cultures, the frequencies of metaphases containing structural aberrations were comparable to concurrent vehicle controls, and frequencies of polyploid metaphases were 0 to 1.0%.

Thus it was concluded that the test chemical induced polyploidy when CHL (V79) cells were treated for 48 hours in the absence of S9, but had no clastogenic effect on DNA.

This study is classified as ACCEPTABLE and satisfies the requirement for FIFRA Test Guideline for *in vitro* cytogenetic data.

COMPLIANCE:

Signed and dated GLP, Quality Assurance and Data Confidentiality statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material: XDE-537 or XRD-537 (cyhalofop-butyl technical)
Description: Off-white powder
Lot/Batch No.: AGR284267
Purity: 97.4% a.i.
Stability of compound: "Stable in a dark cold room"; also storage in "dark, cold room."
Solvent used: Dimethylsulfoxide (DMSO)
Other comments: Solubility in water < 1.0 ppm
Chemically: R-(+)-n-butyl 2 - [4-(2-fluoro-4-cyanophenoxy) phenoxy] propionate. Test material is listed as XDE-537 (title page of the Final Report) and as XRD-537 (throughout the Final Report).

2. Control Materials:
Negative: Growth medium, Eagle's MEM (untreated controls)
Solvent/final concentration: 0.5% DMSO
Positive: Nonactivated (concentration, solvent): Mitomycin-C (MMC), 0.4 μg/mL in Hank's BSS
Activated (concentrations, solvent): Benzo(a)pyrene (Bp), 37.84 μ/mL, in DMSO

3. Activation: S9 derived from 7-week male Sprague-Dawley rats:

x	Aroclor 1254	x	induced	x	rat	x	liver
	phenobarbital		non-induced		mouse		lung
	none						other
	other						other
S9-Mix Composition/10 mL: 8 mM $MgCl_2$, 33 mM KCl, 5 mM Glucose 6-phosphate, 4 mM NADPH, 4 mM NADH, 100 mM Na phosphate buffer, S9 fraction (3mL) and H_2O (7 mL).							

4. Test Compound Concentrations Used (for preliminary cytotoxicity and main assay):
Non-activated conditions: 19.5 to 5000 μg/mL for 24 or 48 hours.

Activated conditions: 19.5 to 5000 μg/mL for 6 hours followed by 18 hours in fresh culture medium.

5. Test Cells: Mammalian cells in culture, [Chinese hamster lung cell line]

Properly maintained: Yes.

Cell line or strain periodically checked for *Mycoplasma* contamination: Yes

Cell line or strain periodically checked for karyotype stability? Yes.

B. TEST PERFORMANCE

1. Preliminary Cytotoxicity Assay: *Relative growth inhibition, compared to* DMSO control (= 100%).

2. Cytogenetic Assay:

a. Cell treatment
Cell exposed to test compound, solvent, or positive control for _ 24, 48 hours (nonactivated), 6 hours (activated), followed by 18 hours in fresh growth medium.

b. Spindle inhibition
Inhibition used/concentration: Colchicine, 0.5 μg/mL

Administration time: 2 hours (before cell harvest)

c. Cell harvest
Cells exposed to test material, solvent or positive control were harvested 0 hours after termination of treatment (nonactivated), 18 hours after termination of treatment (activated).

d. Details of slide preparation: Standardized procedure.

e. Metaphase analysis
Number of cells examined per dose: 200 (100 metaphases per culture)

Scored for structural: Yes.
Scored for numerical: Yes.

If yes, list: polyploidy only.

Coded prior to analysis: Yes.

f. Evaluation criteria: A test substance is judged to be a clastogen when the highest frequency of aberrant metaphases including gaps is 10% or more, with reproducibility, and a dose-related increase is observed. If the frequency is 5% + but less than 10%, clastogenic potential is considered "inconclusive"; "negative" if less than 5%".

g. Statistical analysis: None were reported.

II REPORTED RESULTS

A. Compound Solubility: Compound precipitation was noted at concentrations ≥ 312.5 μg/mL.

B. Preliminary Cytotoxicity Assay: Relative growth rate was > 50% of solvent control at all concentrations ± S9, (Table 1, p. 20). Hence 5 dose levels ranging from 312.5 to 5000 μg/mL were selected for the cytogenetic determination.

C. Cytogenetic Assay:

Although structural chromosome aberrations were observed in XDE-537 treated cells, the frequencies of such aberrant metaphases after 24 or 48 hour treatment were less than 5%, *i.e.*, 1.0 to 3.0% (Table 2, page 21, attached), and 1.5 to 2.5% (Table 3, page 22, attached), *i.e.*, not exceeding the solvent (DMSO) control values. Therefore, it was considered that the compound did not induce structural chromosome aberrations in the absence of S9.

The frequencies of polyploid metaphases were 1.5 to 4.0% after 24 hours of treatment, compared to 0% in positive and solvent controls (Table 2). However, the incidence of polyploid cells was increased to 17.0% and 16.5% at 312.5 and 625 μg/mL, respectively, after 48 hours of treatment, *i.e.*, a positive result. The response was not dose-related with an increase of 0.5% at the HDT. Reexamination of these results was conducted at 7 levels ranging from 39.1 to 2500 μg/mL, again resulting in frequencies of polyploidy of 17.5% and 16.0% at the same dose levels (Table 6, p. 25, attached) after 48 hours, thus confirming the initially positive result for polyploidy induction.

In cell cultures treated in the presence of S9, the frequencies of metaphases with structural aberrations were also less than 5%, and no numerical aberrations were induced (Tables 4, 5, pp. 23 and 24, attached), consistent with historical control data (Appenndix 1, p. 26, attached).

At doses above 1250 μg/mL, the frequency of polyploidy gradually decreased, to 7.5 - 9.0% at 1250 μg/mL, 3 to 6.5% at 2500 μg/mL and 0.5 at 5000 μg/mL. The investigators hypothesize that this decrease was not due to cell toxicity, but rather to precipitation of test substance in culture medium. The sequence presented appears valid since the test substance was suspended uniformly in the medium, and dissolved almost completely during the 24 hour treatment at 312 and 625 μg/mL. At concentrations of 1250, 2500 and 5000, heavy precipitation was observed, and frequencies of polyploidy gradually decreased, comparably at both the 24 and 48 treatments; hence, it is assumed there was not sufficient interaction between cells on the surface of the dish and precipitated test substance floating on the surface of the culture medium; which explains the low incidence of polyploidy at higher doses. Further, mitotic index was lowest at 625 μg/mL (Tables 3 and 6) supporting the above considerations.

Polyploid cells were not induced in the S9-activated phase of testing. This was explained by the investigators due to: (i) too short a treatment period (6 hour, followed by 18 hour fresh medium) compared to continuous 48 hour exposure under nonactivated conditions which apparently induced conditions for polyploidy; and (ii) possible inactivation or detoxification of test substance by the S9 mix.

III. REVIEWER'S DISCUSSION/CONCLUSIONS

A. The reviewer agrees with the conclusions of this study, generated by well recognized procedures. The explanation for presence of weak polyploidy by the direct method (-S9) but not in the presence of S9 seems valid.

B. Study deficiencies: None.

CYHALOFOP-BUTYL

MICRONUCLEUS (84-2)

EPA Reviewer: Irving Mauer, Ph.D. Date: 11/06/00
Registration Action Branch 3, HED (7509C)
EPA Secondary Reviewer: Nancy McCarroll Date: 12/03/00
Toxicology Branch 1, HED (7509C)
01/05/2001
TXR No: 0050348

DATA EVALUATION RECORD

STUDY TYPE: *In vivo* mammalian cytogenetics - micronucleus assay in mice, OPPTS 870.5395 (§84-2)

DP BARCODE: D268553 SUBMISSION CODE: S582655

P. C. CODE: 082583 TOX. CHEM. CODE: None

TEST MATERIAL (PURITY): XDE-537 (cyhalofop-butyl, 97.4% a.i.)

SYNONYMS: R-(+)-n-butyl 2-[4-(2-fluoro-4-cyanophenoxy) phenoxy] propionate; XRD-537

CITATION: Matsumura, H., Matsumoto, K., Kato, T., Watanabe, K. and Ohta, T. (1991). XDE-537: Micronucleus Test in Mice; performed at the Kodaira Laboratories of the Institute of Environmental Toxicology (IET), Tokyo (Japan), Laboratory Study GHF-R 271 (IET 91-0058), dated 26 August 1991. MRID 45000422. Unpublished.

SPONSOR: Dow Elanco Japan Ltd., for Dow AgroSciences LLC, Midland MI

EXECUTIVE SUMMARY: In a mouse bone marrow test (MRID 45000422), XDE-537 (cyhalofop-butyl, 97.4% in aqueous sodium carboxymethylcellulose, CMC) was administered at a dose of 5000 mg/kg once orally to groups of 5 male and 5 female ICR (Crj:CD-1) mice, and bone marrow smears examined 24, 48 and 72 hours after dosing for the presence of micronuclei in polychromatic erythrocytes (mPCE). Alternatively, the test article was administered orally to groups of mice at doses of 1250, 2500 and 5000 mg/kg, and bone marow smears prepared 24 hours after dosing. In addition to mice treated with vehicle (CMC), additional males and females were given mitomycin-C (MMC) to serve as positive controls.

No significant clinical signs or changes in the frequencies of PCE to total erythrocytes were recorded in either test. **No significant increases in the frequencies of mPCE were found in bone marrow cells at harvest of either the time-course or dose-response test.** In contrast, the positive control group (MMC) showed a significant increase in mPCE.

From these results, it is concluded that XDE-537 (cyhalofop-butyl) does not induce micronuclei in the bone marrow cells of ICR mice treated once orally at doses up to the HDT under conditions of this study.

This study is classified as ACCEPTABLE and satisfies the requirement for FIFRA Test Guideline 84-2 for *in vivo* cytogenetic mutagenicity data.

COMPLIANCE: Signed and dated GLP, Quality Assurance and Data Confidentiality Statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material: XDE-537 (cyhalofop-butyl technical)
Description: Off-white powder
Lot/Batch No.: AGR284267
Purity: 97.4% a.i.
Stability of Compound: "Stable in a dark cold room," where stored
Solvent used: 0.5% Aqueous sodium carboxymethylcellulose (CMC)
Solubility: Water < 1.0 ppm
Comment: The test material is referred to as XDE-537 (title page of the final report) and as XRD-537 throughout the report.

2. Control Materials:
Negative/Route of Administration: None
Vehicle/Final Volume/route of Administration: CMC, 20 mL/kg, once orally
Positive/Final Dose(s)/Route of Administration: Mitomycin-C (MMC), 10 mg/kg (0.5 mg/mL) in saline, once orally.

3. Preliminary Toxicity or Range Finder:

Groups of 3 males and 3 females were given XRD-537 at levels of 313, 625, 1250, 2500 or 5000 mg/kg and observed for 72 hours.

Main Tests:

a. Time Course Study

Groups of 5 males and 5 females were given 5000 mg/kg XRD-537 once and bone marrow samples taken at 24, 48 and 72 hours post-dosing.

b. Dose Response Study

Groups of 5 males and 5 females were given single doses of XRD-537 at levels of 1250, 2500 or 5000 mg/kg and bone marrow cells taken 24 hours post-dosing.

4. Test Animals:

a. Species: Mouse. Strain: ICR (Crj:CD-1). Age: 6 weeks.
Weight: Male: (not provided). Female: (not provided).
Source: Charles Rive Japan Inc., Kanagawa.
b. Number of animals used per dose: 5 males; 5 females.
c. Properly maintained? Yes.

B. TEST PERFORMANCE:

1. Treatment and Sampling Times:

a. Test compound:

Test I:

Dosing: 5000 mg/kg: x once ____ twice (24 hours apart)
____ other (describe):

Sampling (after last dose): ____6 hours ____ 12 hours

x 24 hours x 48 hours x 72 hours

Test II

Dosing: 1250, 2500, 5000 mg/kg: once x

Sampling (after last dose): ____6 hours ____12 hours

x 24 hours ____48 hours ____72 hours

b. Negative and/or vehicle control (CMC)

Test I

Dosing: x once ____twice (24 hr. apart) ____other (describe)

Sampling (after last dose): ____6 hours ____12 hours
x 24 hours x 48 hours x 72 hours (Tests I and II)

Test II

Dosing 0,0,0

Sampling (after last dose) ___6 hours ___12 hours __x__24 hours ___48 hours ____72 hours

c. Positive Control (MMC)

Tests I and II

Dosing: ___x___once _____twice (24 hours apart)
_____other (describe):

Sampling (after last dose): _____6 hours _____12 hours ___x___ ___x__24 hours _____48 hours _____72 hours (Tests I and II)

2. Tissues and Cells Examined: Polychromatic erythrocytes (PCE) plus normochromatic erythrocytes (NCE).

___x___bone marrow
other (list): None

Number of polychromatic erythrocytes (PCE) examined per animal: 1000

Number of normochromatic erythrocytes (NCE; more mature RBCs) examined per animal: among 1000 NCE plus PCE.

3. Details of Slide Preparation: Standardized procedures for bone marrow cells, spread on microscope slides, and stained with 2% Giemsa.

4. Statistical Methods: The frequencies of micronucleated polychromatic erythrocytes (mPCE), F, are shown as percentages of PCE with micronuclei among 1000 PCE[1]. PCE ratios (R) are shown as percentages

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[1] $F(\%) = \frac{mPCE}{PCE} \times 100$

of PCE amoung 1000 erythrocytes (sum of PCE + NCE)[2]. Incidences of mPCE analyzed using tables of Kastenbaum and Bowman (1970). Jonckheere's Trend Test was used to determine dose-response , and PCE ratios by Wilcoxon's Sum of Ranks Test.

5. Evaluation Criteria: Criteria for distinguishing micronuclei and staining differentiation of PCE and NCE were presented. The above mentioned statistical methods were applied to evaluate the results of assaying the presence and significance of micronucli in treated mice.

II REPORTED RESULTS

A. PRELIMINARY TOXICITY ASSAY:

No animals died within 72 hours of treatment at 5000 mg/kg; and no clinical adverse signs were observed (Appendices 1 and 6, MRID 45000422, pages 16 and 21). Hence the maximum dose selected was 5000 mg/kg for both sets of tests.

B. MICRONUCLEUS ASSAYS:

Tests a and b: In agreement with the above findings, no deaths or clinical signs were observed. There were also no significantly increased frequencies of mPCE at any dose in either the time-course or dose-response studies (Tables 1 and 2, MRID 45000422, pages 14 and 15, attached), whereas a significant increase ($p < 0.01$)was registered for MMC-treated animals.

In the animals treated with XDE-537 neither test recorded changes in the ratio of PCE:NCE. Hence, the investigators concluded that a single administration of XDE-537 did not induce micronuclei in bone-marrow cells of ICR mice at conditions under which this study was conducted.

III. REVIEWER'S DISCUSSION/CONCLUSIONS

Since no changes in the PCE/NCE ratio were recorded, in addition to no clinical effects of the test compound in CMC, the suspicion arose as to whether the test compound dissolved in CMC reached the bone marrow in sufficient concentration to exert any effect

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[2] $R(\%) = \frac{PCE}{PCE + NCE} \times 100$

(cytotoxic, or cytogenetic). MMC-treated mitotic indices were significantly depressed in the time course study but not in the dose-response experiment; in both tests, however, significantly increased mPCE values were recorded at low doses (10 mg/kg) compared to XDE-537 treatment (up to 5000 mg/kg). Hence XDE could be a weak mutagen, especially when dissolved in CMC, preventing full expression of its potential cytogenetic activity, compared to the freer transport of a strong clastogen (MMC) dissolved in saline.

IV DEFICIENCIES: None.

CYHALOFOP-BUTYL

BACTERIAL REPAIR (84-2)

EPA Reviewer: Irving Mauer, Ph.D. Date: 11/06/00
Registration Action Branch 3, HED (7509C)
OPP Secondary Reviewer: Nancy McCarroll Date: 12/03/00
Toxicology Branch 1, HED (7509C)
TXR No: 0050348

01/05/2001

DATA EVALUATION RECORD

STUDY TYPE: Repair in *Bacillus subtilis* H17 (*rec*$^+$) and M45 (*rec*-), OPPTS 870.5500

DP BARCODE: D268553 (sub-bean to D267558) SUBMISSION CODE: S582655

P.C. CODE: 082583 TOX. CHEM. NO.: None

TEST MATERIAL (PURITY): XDE-537 (cyhalofop-butyl, 97.4% a.i.)

SYNONYMS: R- (+) - n - butyl 2 - [4 - (2 - fluoro - 4 - cyanophenoxy) phenoxy] propionate

CITATION: Watanabe, K., Kato T. and Ohta, T. , (1991). XDE-537 BE DNA Repair Test, performed at the Kodraira Laboratories of the Institute of Environmental Toxicology, Kodaira, Tokyo, (Japan), Laboratory Study No. GHF-R 272 (IET 90-0176), dated 15 March 1991. MRID 45000420. Unpublished.

SPONSOR: Dow Elanco Japan Ltd., Tokyo, Japan, for Dow AgroSciences LLC, Midland MI

EXECUTIVE SUMMARY: In a bacterial DNA repair test (MRID 45000420), cultures of *Bacillus subtilis H17* (*rec*$^+$) and *B. subtilis M45* (*rec*$^-$) were exposed to cyhalofop-butyl as XDE-537 (97.4% a..i. in dimethylsulfoxide) in the presence and absence of metabolic activation at dose levels of 200 to 10,000 μg/disk, and comparative inhibitory zones determined after incubation at 37° C for 24 hours. Kanamycin served as the negative control, and mitomycin-C and 2-aminoanthracene as positive controls for tests without (-S9) and with (+S9) metabolic activation, respectively.

No differences in any inhibitory zone in either strain was found up to the highest dose tested (HDT) ± S9. Both the negative and positive controls induced the expected responses. Hence the test article is considered negative in this test.

This study is classified as ACCEPTABLE and satisfies the FIFRA Test Guideline 84-2 for bacterial repair data.

COMPLIANCE: Signed and dated Quality Assurance, GLP and Data Confidentiality Statements were provided.

MATERIALS AND METHODS

A. MATERIALS

1. Test Material: XDE-537 (cyhalofop-butyl technical)
   Description: Off-white powder
   Lot/Batch No.: AGR284267
   Purity %: 94.7% a.i.
   Stability: "Stable in a dark, cold room," storage therein also
   Solubility: Water < 1.0 ppm
   Solvent: Dimethylsulfoxide (DMSO)

2. Control Materials
   Negative: Kanamycin, 0.2 μg/disk
   Solvent: DMSO
   Positive: Nonactivation (-S9): Mitomycin C (MMC), 0.01 μg/disk in DMSO
   Activation (+S9): 2-Aminoanthracene (2-AA), 20 μg/disk in DMSO.

3. Test Organisms: *Bacillus subtilis*

   Recombination - wild strain (*rec*$^+$) H17
   Recombination - deficient strain (*rec*$^-$) M45

   Source: Dr. T. Kada, National Institute of Genetics, Mishima (Japan)

4. Preparation of Spores: Bacterial cells were grown in B2 medium (1% meat extract, 1% polypeptone, 0.5%NaCl, pH7), inoculated to a modified Schaeffer's medium and incubated at 37°C for 3 to 5 days, with shaking. Cells were washed with buffer, then treated with lysozyme and sodium dodecyl sulfate (SDS). Spores were suspended in sterile water at 4° C; spores of M45 and H17 prepared 2 days apart were used in this experiment.

   The characters of the tester strains were checked at the time of spore preparation by ultraviolet sensitivity, and response to a positive control chemical and a negative control (details provided in an appended report).

5. Preparation of S9 Faction: S9 was derived from livers of male Sprague-Dawley rats given a single 500 mg/kg i.p. injection of Aroclor 1254, and perfused livers homogenized and 9000 g. superantant fraction

(microsomal) stored at -80°C until use (fraction prepared as Lot 70, protein content = 26.5 mg/mL used in this experiment).

The activity of this fraction was checked by mutgenicity of 7, 12-dimethylbenz(a)anthracene against *Salmonella typhimurium* TA100 and T98.

Co-factor Mix:

$MgCl_2$	8 mM
KCl	33 mM
G-6-PO_4	20 mM
NADP PH	40 mM
NADH	40 mM
Sodium Phosphate Buffer	PH 7.4
S9 Frction	Lot 70; protein content: 26.5 mg/mL

6. Dose Levels: The test agent, XDE-537, was dissolved in DMSO at a concentration of 500 mg/mL, and 6 dose levels were selected for testing: 200, 500, 1,000, 2,000, 5,000 and 10,000 μg disk.

7. Procedure (*rec*⁻ assay): In empty 90-mm Petri dishes, 0.1 mL of spore solution ($3x10^7$ spores/mL) of the tester strains with/without 0.05 mL of S9 fraction was covered with 0.08% molten B2 agar medium held at 45°C and mixed uniformly. Paper-filter disks (8 mm diameter and 1 mm thick) were soaked with 20 μl of test substance solutions; for metabolic activation experiments, an additional 20 μl of co-factors plus S9 were added to each dish. Disks were placed on the prepared spore-agar plates(in duplicate). The diameters of inhibitory zones for each strain were measured after incubation at 37° C for 24 hours.

8. Criteria: Results were considered positive when reproducible differences in diameter of growth inhibitory zones between strains were 5 mm or more

at dose levels that caused inhibitory zones of 0 to 4 mm in diameter in the H17 (*rec.*$^{+}$) strain.

9. Reported Results: XDE-537 did not cause any inhibitory zones in either strain, M45 (*rec*$^{-}$) or H17 (*rec*$^{+}$), at doses up to the HDT, 10,000 μg/disk, ± S9 (Table 1, page 9 of MRID 45000420 attached). By contrast, the negative control (Kanamycin) caused approximately equivalent inhibition of repair deficient M45 versus repair competent H17 while the positive controls (MMC-S9; 2A +S9) induced preferential inhibition of strain M45 as compared to H17. Hence, the investigators considered the test article article to be negative in this bacterial DNA repair test under conditions used in this experiment.

III. REVIEWERS' DISCUSSION/CONCLUSIONS

This reviewer agrees with the conclusions of this bacterial DNA repair experiment ~~conducted under conditions employed that the test chemical is negative for bacterial~~ repair in *B. subtilis*.

IV STUDY DEFICIENCIES: None.

DATA EVALUATION RECORD

CYHALOFOP BUTYL
(DE-537-*n*-butyl ester)

STUDY TYPE: ACUTE NEUROTOXICITY - RAT [OPPTS 870.6200 (§81-8)]
MRID 45000409

Prepared for

Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
1921 Jefferson Davis Highway
Arlington, VA 22202

Prepared by

Chemical Hazard Evaluation Group
Toxicology and Risk Analysis Section
Life Sciences Division
Oak Ridge National Laboratory
Oak Ridge, TN 37831
Task Order No. 01-81FF

Primary Reviewer: Sylvia S. Talmage, Ph.D., D.A.B.T.	Signature: Date:	Sylvia S. Talmage JAN 26 2001
Secondary Reviewers: Carol S. Forsyth, Ph.D., D.A.B.T.	Signature: Date:	Carol S Forsyth JAN 26 2001
Robert H. Ross, Group Leader	Signature: Date:	Robert H. Ross JAN 26 2001
Quality Assurance: Lee Ann Wilson, M.A.	Signature: Date:	L. A. Wilson JAN 26 2001

Disclaimer

This review may have been altered subsequent to the contractor's signatures above.

Oak Ridge National Laboratory, managed by UT-Battelle, LLC, for the U.S. Department of Energy under contract number

CYHALOFOP BUTYL, TECHNICAL **Acute Neurotoxicity [OPPTS 870.6200 (81-8)]**

EPA Reviewer: John Whalan
Registration Action Branch 2 (7509C)
EPA Work Assignment Manger: S. Williams-Foy, D.V.M.
Registration Action Branch 2 (7509C)
TXR No: 0050348

John Whalan, Date 5-24-01
, Date 5/31/01

DATA EVALUATION RECORD

STUDY TYPE: Acute Neurotoxicity Study - Rat; OPPTS Number: [870.6200 (§81-8)]

DP BARCODE: D268553
P.C. CODE: 082583

SUBMISSION CODE: None
TOX. CHEM. NO.: None

TEST MATERIAL (PURITY): Cyhalofop butyl (97.3%)

SYNONYMS: DE-537 n-butyl ester; DE-537NBU; XRD-537; (R)-2-(4-(4-cyano-2-fluorophenoxy) phenoxy) propanoic acid n-butyl ester

CITATION: Mattsson, J.L., McGuirk, R.J., Johnson, K.A. (1998) DE-537 n-butyl ester: acute neurotoxicity study in Fischer 344 rats. The Dow Chemical Company, Health & Environmental Research Laboratories, Midland, MI 48674. Laboratory Project Study ID 980022, June 23, 1998. MRID 45000409. Unpublished.

SPONSOR: Dow AgroSciences LLC (DAS), 9330 Zionsville Rd., Indianapolis, IN 46268.

EXECUTIVE SUMMARY: In an acute oral neurotoxicity study (MRID 45000409), DE-537 *n*-butyl ester (Lot # AGR295713, 97.3% purity) was administered by gavage to 10 male and 10 female Fischer 344 rats at single doses of 0, 200, 600, or 2000 mg/kg. Body weights were recorded pre-exposure and on test days 1, 2, 8, and 15. Functional observational battery (FOB) and motor activity tests were preformed pretreatment, on the day of test material administration (day 1), and on days 8 and 15 post-treatment. At the completion of the study (day 16), 5 rats/sex in the control and high-dose groups were euthanized and perfused in situ for neuropathological examination. Tissues of the central and peripheral nervous system were examined microscopically.

There were no mortalities, no clinical signs, and no effects on body weight at any time following treatment. After 15 days, body weights of all treated groups were ≥95% of the respective control values for males and females. There were no effects of treatment on FOB parameters or motor activity. There were no histopathologic findings in the brain or tissues of the central or peripheral nervous system attributable to treatment.

The NOAEL for male and female rats is ≥2000 mg/kg based on the absence of clinical signs, a lack of effects on FOB parameters and motor activity, and the absence of neuropathologic lesions. A LOAEL was not identified.

This study is considered **Acceptable/Guideline** as an acute oral neurotoxicity study and fulfills FIFRA guideline requirements for an acute oral neurotoxicity study in rats **[OPPTS 870.6200 (§82-1b)]**.

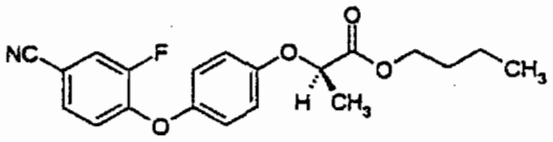
COMPLIANCE: Signed and dated GLP, Quality Assurance, Flagging, and Data Confidentiality statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: Cyhalofop butyl (DE-537 *n*-butyl ester)

Description: off-white solid
Lot No.: AGR295713
Purity: 97.3%
Stability of compound: stable for 13 days
CAS #: 122008-85-9
Structure:



2. Vehicle and/or positive control

The test material was administered by gavage in 0.5% aqueous methyl cellulose. Treatment of the control group was not described. Animals were fasted prior to dosing.

3. Test animals

Species: Rat
Strain: Fischer 344
Age and weight at study initiation: 6-8 weeks old; males: 94.8-127.8 g; females: 64.7-84.1 g
Source: Charles River, Raleigh, NC
Housing: individually in stainless steel cages with wire mesh floors
Diet: Animals were fed Purina Certified Rodent Lab Diet #5002, *ad libitum*; food was withheld pretest during a fasting period and during neurotoxicity testing
Water: Drinking water (tap) was available *ad libitum*.
Environmental conditions:
Temperature: 21.8-22.2°C
Humidity: 48-51%
Air changes: 12-15/hour
Photoperiod: 12 hour light/12 hour dark
Acclimation period: 1-3 weeks

B. STUDY DESIGN

1. In life dates

Start: February 24, 1998; end: March 20, 1998

The starting day was staggered over 4 days based on the need to handle smaller groups of animals for the neurotoxicity tests. Animals were divided into four subsets of 20 rats each which were counterbalanced over the different dose levels and sexes. Animals were observed for 2 weeks post dosing.

2. Animal assignment

Animals were stratified by body weight and then assigned to the test groups in Table 1 by means of a computerized randomization procedure.

TABLE 1: Study design

Test group	Dose (mg/kg)		Number of animals	
	Males	Females	Males	Females
1 (control)	0	0	10	10
2 (low-dose)	200	200	10	10
3 (mid-dose)	600	600	10	10
4 (high dose)	2000	2000	10	10

Data taken from pp. 12 and 13, MRID 45000409.

3. Dose selection rationale

Doses were based on a previous acute oral gavage study in which male and female rats were administered 2000 mg/kg (the limit dose for OPPTS 870.6200 guideline) in 0.5% methyl cellulose. There were no treatment-related clinical signs during the eight hours after dosing. One female had urine soiling on the day following dosing. Therefore, 2000 mg/kg was selected as the high dose in the present study and the mid- and low-dose were reductions of the high-dose by factors of approximately 3 and 6, respectively. These doses were selected to provide dose-response information and to ensure a NOAEL. Since no time of peak effect could be ascertained, a time of between six and eight hours after dosing was arbitrarily chosen as the time to conduct the FOB and motor activity test. The same time of day was used for the tests on days 8 and 15.

4. Test material preparation and analysis

Preparation and sampling of the test material were described in previous reports that were not provided. The test material was dissolved in 0.5% aqueous methyl cellulose. The dosing volume was 10 mL/kg.

For homogeneity, three aliquots (top, middle and bottom) were taken from each of the 200 (20 mg/mL) and 2000 mg/kg (200 mg/mL) dose containers. The stability of the test material in these two solutions was confirmed by analyzing samples on days 1 and 13; storage conditions were not described in the present study. The concentrations in a single sample of each dose level (0, 200, 600, and 2000 mg/kg) were analyzed.

Results -

Homogeneity analysis: The concentrations of the three aliquots of the 20 mg/mL solution averaged 19.8±0.8 mg/mL and the mean concentration in the three aliquots of the 200 mg/mL solution was 206±2 mg/mL. The relative standard deviations were 4.04 and 0.97%, respectively.

Stability analysis: For the 20 and 200 mg/mL solutions, percents of the initial day solutions were 101 and 100%, respectively on day 13. Observed concentrations for the two solutions on days 1 and 13 were 19.8 and 19.9 mg/mL and 206 and 207 mg/mL, respectively.

Homogeneity and stability of ^{14}C-labeled DE-537 *n*-butyl ester were also determined in an earlier study (MRID 45000528) in which the radioactive label was added to the α or β ring of 0.5 and 25 mg/L solutions. For homogeneity analyses, coefficients of variation (C.V.) of solutions in gavage syringes ranged from 0.1 to 17.5% (one sample); all other C.V values were less than 10%. The radiochemical purity of the four radio-labeled solutions (0.5 and 25 mg/mL solutions labeled in the α or β rings), did not decline over 21 days. On the 21st day, radiochemical purities of the four solutions ranged from 97.7-99.0%. Stability of the non-radiolabeled solutions, determined by HPLC/UV detection was 100% at all time points

Concentration analysis: The percent of target concentrations of DE-537 *n*-butyl ester in the 200, 600, and 2000 mg/kg dose levels were 99, 101, and 103, respectively.

The reported analytical data indicated that the mixing procedure was adequate, that the dosing solutions were stable for 13 days and that the variance between targeted and actual dosage to the animals was acceptable.

5. Statistics

FOB observations were ranked and the incidences of ranked observations between control and each treated group, for each sex, were evaluated by a z-test of proportions at $\alpha = 0.02$. If more than one rank within the same observation had significant z scores, the toxicologically most significant rank was reported. For all continuous data, means and standard deviations were calculated and homogeneity of variance was evaluated with the Bartlett's test ($p = 0.01$). In order to analyze data based on two sexes and four data collection times (preexposure and days 1, 8, and 15), repeated-measures ANOVAs were initially employed. The multivariate index was the Pillai Trace statistic. Data analyzed by the repeated-measures ANOVA were body weight, grip strength, body temperature, landing foot splay, and motor activity. The following

interactions were evaluated: (1) treatment x time, (2) treatment x time x sex, and (3) treatment x time x subsession (motor activity only). For continuous data, the error rate was set at 0.05.

C. METHODS

1. Observations

All animals were observed cage side once/day for clinical signs of toxicity and mortality except on days when clinical examinations were performed. Clinical examinations were performed on all animals on test days 2, 3, and 4. Clinical examinations involved evaluations of the skin, fur, mucous membranes, respiration, nervous system function (tremors and convulsions), swelling, masses, and behavior.

2. Body weight

All animals were weighed pre-exposure and on test days 1, 2, 8, and 15.

3. Food consumption and compound intake

Food consumption was not determined. Compound intake was based on gavage doses.

4. Functional observational battery (FOB)

The FOB was conducted after the motor activity test. All animals/sex/group were subjected to a baseline FOB one week prior to treatment. The FOB was repeated at six to eight hours following administration of the dose (time of peak activity could not be ascertained), and again at 8 and 15 days following treatment. Testing was staggered over 4 days based on the need to handle smaller groups of animals for the neurotoxicity tests. Animals were divided into four subsets of 20 rats each which were counterbalanced over the different dose levels and sexes. The following FOB observations were performed by a trained technician who was blind to the treatment of the animals; except for categorical observations, these observations were ranked with rankings ranging from 1-4 or 1-5:

a. Home cage observations

Abnormal movements or behavior; resistance to removal from cage.

b. Observations during handling

Palpebral closure, lacrimation, pupil size, pupil response to light, salivation, muscle tone, extensor-thrust response, reactivity to handling.

c. Open field observations

Activity (ambulatory and rearing; 1 minute); responsiveness to noise, touch, and tail pinch; gait evaluation; urination, fecal pellets.

d. Sensorimotor and neuromuscular measurements

Hindlimb and forelimb grip strength, and landing foot splay.

e. Other

Body temperature. In addition, the following categorical observations (ranked yes/no) were made at any time during the FOB: abnormal skin or hair-coat, abnormal behavior, abnormal respiration, abnormal muscle movements, abnormalities of the eye, abnormal urine or feces, excessive soiling, general abnormalities, abnormal posture.

Details of the environmental conditions during the FOB evaluations were not provided. The FOB was performed by a single technician who was blind to the treatment status of the animals.

5. Motor activity

Motor activity was measured at six hours post-dosing (in the afternoon of each day) and prior to each FOB session. Motor activity was monitored for 48 minutes (six 8-minute sessions) in one of 24 circular chambers bisected by an infrared photobeam. Motor activity was measured as the number of beam interruptions that lasted more than 100 milliseconds and followed an interval between beam breaks that was greater than 100 milliseconds (to discount activity such as tail flicking or head bobbing). Motor activity was monitored with a computerized system. Data were presented as square roots of total counts.

6. Sacrifice and pathology

On day 16, five randomly selected rats/sex/dose were injected with heparin and anesthetized with methoxyflurane. Rats were perfused via the left ventricle with phosphate buffer containing sodium nitrite followed by a phosphate-buffered solution of 1.5% glutaraldehyde and 4% formaldehyde. Major organs and tissues were examined grossly and preserved. Tissues from the central nervous system, spinal cord, and muscles were embedded in paraffin, sectioned approximately 6 μ thick, and stained with hematoxylin and eosin. Spinal nerve roots, dorsal root ganglia, and peripheral nerves were osmicated, embedded in plastic (epoxy resin), sectioned, and stained with toluidine blue. The checked (X) tissues from the control and high-dose groups were examined microscopically:

X	CENTRAL NERVOUS SYSTEM	X	PERIPHERAL NERVOUS SYSTEM
	BRAIN		**PERIPHERAL NERVES**
X		X	
	Olfactory bulb	X	Sciatic
X	Forebrain	X	Sural
	Cerebrum		Tibial
X	(frontal, parietal, temporal, occipital)		Peroneal
X	Midbrain	X	**OTHER**
X	Cerebellum	X	
X	Pons	X	Lumbar dorsal root ganglion
X	Medulla oblongata	X	Lumbar dorsal root fibers
X	Thalamus/hypothalamus	X	Lumbar ventral root fibers
	Pituitary	X	Cervical dorsal root ganglion
			Cervical dorsal root fibers
X	**SPINAL CORD**	X	Cervical ventral root fibers
X		X	
	Cervical swelling		Gastrocnemius muscle
	Lumbar swelling		Anterior tibial muscle
	Thoracic swelling		
X	**OTHER**		
X			
X	Gasserian ganglion		
	Trigeminal nerves		
	Optic nerves		
	Eyes		

The remaining animals that survived to term were sacrificed by carbon dioxide asphyxiation; additional tissues were not collected.

6. Positive and Historical Control Data

Positive control data providing evidence of the observational methods to detect major neurotoxic endpoints were presented in Appendix C of the study report. Positive control substances included chlorpromazine, d-amphetamine sulfate, and atropine plus physostigmine sulfate; saline was the negative control substance. Head weaving and piloerection were observed in rats treated with d-amphetamine. Chlorpromazine-treated rats held fixed postures and atropine-physostigmine treated rats had tremors and a decreased response to tail pinch. Data were also presented for body temperature, grip strength, and foot splay. Data were presented for a single technician who also made the observations in the present study.

Positive control data showing an increase and decrease in activity in rats treated with amphetamine and chlorpromazine, respectively, were presented in Appendix B of the study report. The historical data from control rats showed that habituation was reached during the 5th-6th 8-minute subsession.

Positive control data for neuropathology were presented in Appendix D of the study report. Peripheral nerve degeneration and tumors of the central nervous system were demonstrated in rats chronically administered acrylamide in the drinking water.

Extensive information in the form of a published journal article on spontaneous lesions of the nervous system (historical control data) of male and female Fischer 344 rats was also presented in Appendix D of the study report.

II. RESULTS

A. CLINICAL OBSERVATIONS AND MORTALITY

There were no deaths. No treatment-related effects were observed in any rats at any time during the study. Non-treatment-related fecal soiling was observed in one male control rat on test days 2 and 3 and urine soiling was observed on one control and one low-dose female on test day 2 only.

B. BODY WEIGHT AND WEIGHT GAIN

Group mean body weights and total body weight gains are shown in Table 2. There were no effects of treatment on body weights throughout the observation period. Final body weights of treated males and females were 95-99% and 97-99% of the respective control groups. Total weight gains ranged from 88 to 98% of the control weight gain for males and 94-97% of the control weight gain for females.

TABLE 2. Group mean body weights (g) for selected days and total body weight gains (g) of rats administered an acute oral dose of DE-537 *n*-butyl ester[a]

Males				
Day of study	Dose (mg/kg)			
	0	200	600	2000
Pretreatment	111±7.7	110±8.3	110±7.6	113±6.9
1	130±10.5	127±11.1	128±10.7	130±7.4
8	152±13.8	148±13.8	151±15.5	154±9.7
15	169±15.9	161±13.9 (95)	167±21.5 (99)	167±10.7 (99)
Total weight gain[b]	58	51 (88)	57 (98)	54 (93)
Females				
Day of study	Dose (mg/kg)			
	0	200	600	2000
Pretreatment	74±2.8	73±5.4	75±5.7	74±3.8
1	84±2.7	84±6.2	85±5.9	84±4.1
8	98±4.0	98±6.6	99±6.8	99±6.1
15	109±4.5	106±5.9 (97)	108±7.5 (99)	108±7.1 (99)
Total weight gain[b]	35	33 (94)	33 (94)	34 (97)

Data taken from Table 19, p. 82, MRID 45000409.
[a]Percent of control value in parenthesis.
[b]Calculated by reviewer.

C. FUNCTIONAL OBSERVATIONAL BATTERY (FOB)

Because of the large number of pair-wise statistical comparisons of the ranked data (4 time periods, 16 observations, 2 sexes, and 3 control vs treatment comparisons), there were several false positive findings (ranked differences of 0.5 flagged). For example, one comparison was flagged preexposure: the average rank of urination in females during the preexposure observation was 1.0 in the control group and 1.5 in the 2000 mg/kg group. Following treatment, ten additional comparisons differed by 0.5 or more. On day 1, resistance to removal in females was ranked 3.0 in the control group and 2.5 in the 200 mg/kg group. Ranks were similar among the control and mid- and high-dose groups which indicated a lack of dose-response. On day 8, ranks of resistance to removal between the male control and high-dose groups and urination between the male control and low-dose groups differed by ≥ 0.5. However, none of these differences on days 1 and 8 was significant ($p<0.02$). On day 15, differences in rankings of several parameters were significant ($p<0.02$), but the data did not show a dose-response effect (i.e., all significant differences were between the control and low-dose group or the control and mid-dose group). Because of the lack of a dose-response relationship, these differences in FOB rankings among the groups were not considered treatment-related. It should be noted that in all cases, behavior and gait did not differ among groups and were considered within normal limits. There were no treatment-related effects for either sex at any time in forelimb and hindlimb grip strength, landing foot splay, or rectal temperature.

D. MOTOR ACTIVITY

Motor activities for the total 48-minute sessions on the day of treatment (day 1) and days 8 and 15 were not affected by treatment at any time for either sex (Table 3), i.e., there was no variation between treatment x day, treatment x day x sex, or treatment x day x subsession. Motor activity decreased across the six subsessions for all groups and data for the six subsessions of the male and female control group showed that habituation was reached over the 48-minute test period.

TABLE 3. Motor activity of rats administered an acute oral dose of DE-537 *n*-butyl ester by gavage[a]

Day of study	Dose (mg/kg)			
	0	200	600	2000
Males				
Pretreatment	7.68±1.56	7.55±1.52	7.10±1.34	7.99±1.64
1	7.23±1.80	6.90±1.22	7.04±1.46	7.12±1.82
8	8.85±2.41	7.34±1.03	7.92±1.66	7.72±1.99
15	9.15±2.52	8.39±2.47	7.60±1.34	8.80±0.75
Females				
Pretreatment	9.65±1.97	10.44±3.11	9.80±3.19	11.35±2.19
1	8.80±1.68	8.23±1.75	8.79±2.32	7.80±2.76
8	8.83±2.09	8.47±2.46	8.35±2.48	8.05±0.89
15	9.44±1.56	10.41±3.07	10.85±2.06	11.72±1.90

Data taken from Table 29, p. 92, MRID 45000409.
[a]Values are square roots of total counts.

E. SACRIFICE AND PATHOLOGY

1. Organ weight

No organs were weighed.

2. Gross pathology

No treatment-related gross lesions were observed. One male in the intermediate dose group had a decreased testes and prostate size and one female in the same treatment group had a hiatal hernia of the liver.

3. Neuropathology

No treatment-related lesions of the central or peripheral nervous system were observed. Very slight multifocal degeneration of individual nerve fibers of the trapezoid body of the medulla oblongata were observed in 3 males in the control group, 1 male in the 2000 mg/kg dose group, 3 females in the control group, and 3 females in the 2000 mg/kg dose group. The study authors describe these as common spontaneous lesions of the Fischer 344 rat. These lesions were present in equal or greater frequency in the control group than in the high-dose group. Individual rats had slight degeneration of isolated nerve fibers at other sites: cervical dorsal root ganglion (male, control), lumbar dorsal root ganglion (female, control), proximal sciatic (female, control), tibial (male, 2000 mg/kg), and spinal

cord/cervical (female, 2000 mg/kg). These isolated incidences were not considered treatment-related.

III. DISCUSSION

A. DISCUSSION

Acute administration of DE-537 *n*-butyl ester to male and female Fischer 344 rats at doses up to the limit dose (2000 mg/kg) had no effects on body weight, behavior (as determined by FOB and motor activity evaluations), or the central or peripheral nervous system (as determined by neuropathologic evaluations). The absence of clinical signs immediately following and during the two-week period after treatment precluded identification of a time of peak effect. Thus, the FOB and motor activity tests were arbitrarily performed at 6-8 hours after treatment. The reviewer agrees with this choice of time. The statistical evaluation procedure chosen by the study author resulted in a number of false positive findings in the FOB, but the lack of any dose-response relationship made it clear that positive findings were not treatment related.

This study closely followed OPPTS 870.6200 guidelines. The appropriate tests were described and performed and the results were properly reported. Historical and positive control data and the ability of the technician to detect behavioral effects and of the pathologist to detect neuropathologic effects were demonstrated. Not all of the positive and historical control information was dated, but most of it was performed within the last 10 years. Although the positive and historical control data were not collected concurrent with the present study, the data presented which includes published journal articles, clearly demonstrates the ability of the testing laboratory to detect neurotoxic endpoints.

Based on the lack of effects seen in this study, the NOAEL was ≥2000 mg/kg; a LOAEL was not identified.

B. STUDY DEFICIENCIES

No study deficiencies were identified.

DATA EVALUATION RECORD

CYHALOFOP BUTYL
(DE-537 NBU)

STUDY TYPE: SUBCHRONIC NEUROTOXICITY - RAT [OPPTS 870.6200)82-7)]
MRID 45000509

Prepared for

Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
1921 Jefferson Davis Highway
Arlington, VA 22202

Prepared by

Chemical Hazard Evaluation Group
Toxicology and Risk Analysis Section
Life Sciences Division
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Oak Ridge, TN 37831
Task Order No. 01-81GG

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Disclaimer

This review may have been altered subsequent to the contractor's signatures above.

Oak Ridge National Laboratory, managed by UT-Battelle, LLC, for the U.S. Department of Energy under contract number DE-AC05-00OR22725.

CYHALOFOP BUTYL, TECHNICAL — Subchronic Neurotoxicity [OPPTS 870.6200 (82-7)]

EPA Reviewer: John Whalan, Date 5-25-01
Registration Action Branch 2 (7509C)
EPA Work Assignment Manger: S. Williams-Foy, D.V.M., Date 5/31/01
Registration Action Branch 2 (7509C)
TXR No: 0050348

DATA EVALUATION RECORD

STUDY TYPE: Subchronic Neurotoxicity Study - Rat; OPPTS Number: [870.6200 (§82-7)]

DP BARCODE: D268553
P.C. CODE: 082583

SUBMISSION CODE: None
TOX. CHEM. NO.: None

TEST MATERIAL (PURITY): Cyhalofop butyl (97.1%)

SYNONYMS: DE-537 n-butyl ester; DE-537 NBU; XRD-537; (R)-2-(4-(4-cyano-2-fluorophenoxy) phenoxy) propanoic acid n-butyl ester

CITATION: Johnson, K.A., Shankar, B.E. (1999) DE-537 NBU: 13-week neurotoxicity study in Fischer 344 rats. The Dow Chemical Company, Health & Environmental Research Laboratories, Midland, MI 48674. Laboratory Project Study ID 981113, February 19, 1999. MRID 45000509. Unpublished.

SPONSOR: Dow AgroSciences LLC (DAS), 9330 Zionsville Rd., Indianapolis, IN 46268.

EXECUTIVE SUMMARY: In a subchronic neurotoxicity study (MRID 45000509), DE-537 NBU (Lot # AGR295713, 97.1% purity) was administered in the diet to 10 male and 10 female Fischer 344 rats at doses of 0, 2, 20, or 75 mg/kg/day (males) or doses of 0, 20, 75, or 250 mg/kg/day (females) for 13 weeks. The test article was administered at dietary concentrations adjusted weekly to maintain a constant dose. Body weights, food consumption, and clinical signs were recorded weekly. Neurobehavioral assessment [functional observational battery (FOB) and motor activity testing] were preformed on all rats/sex/group pretreatment and during weeks 2, 4, 8, and 13. At study termination, 5 rats/sex in the control and high-dose groups were euthanized and perfused in situ for neuropathological examination. Tissues from the central and peripheral nervous system were examined microscopically.

There were no mortalities and no treatment-related clinical signs at any time during treatment. Body weights and food consumption were unaffected by treatment. There was no effect of treatment on FOB parameters or motor activity. There were no histopathologic findings in the brain or tissues of the central or peripheral nervous system that could be attributed to treatment.

The NOAELs for male and female rats are ≥75 and ≥250 mg/kg/day, respectively, based on the absence of clinical signs, lack of effects on FOB parameters and motor activity, and absence of neuropathologic lesions. LOAELs were not identified.

This study is considered **Acceptable/Guideline** as a subchronic neurotoxicity study and fulfills FIFRA guideline requirements for a subchronic neurotoxicity study in rats **[OPPTS 870.6200 (§82-7)]**. Although LOAELs were not achieved, the doses were selected based on mild systemic effects at similar doses in an earlier subchronic feeding study.

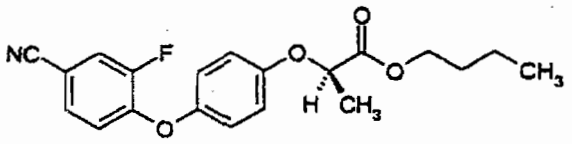
COMPLIANCE: Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: Cyhalofop butyl (DE-537 NBU)

   Description: off-white powder
   Lot No.: AGR295713
   Purity: 97.1%
   Stability of compound: stable in the diet for 32 days
   CAS #: 122008-85-9
   Structure:



2. Vehicle and/or positive control

   The test material was administered in the diet; acetone was used to disperse the test material.

3. Test animals

   Species: Rat
   Strain: Fischer 344
   Age and weight at study initiation: 6 weeks old; males: 93.5-114.8 g; females: 72.9-82.6 g
   Source: Charles River, Raleigh, NC
   Housing: individually in stainless steel cages with wire mesh floors
   Diet: Animals were fed Purina Certified Rodent Lab Diet #5002 in meal form, *ad libitum*
   Water: Drinking water (tap) was available *ad libitum*.
   Environmental conditions:
   Temperature: 19-25°C
   Humidity: 40-70%
   Air changes: 12-15/hour

Photoperiod: 12 hour light/12 hour dark
Acclimation period: 1 week

B. STUDY DESIGN

1. In life dates

Start: July 28, 1998 (preexposure testing); end: November 5, 1998

2. Animal assignment

Animals were stratified by body weight and then assigned to the test groups in Table 1 by means of a computerized randomization procedure.

TABLE 1: Study design

Test group	Dose (mg/kg/day)		Number of animals	
	Males	Females	Males	Females
1 (control)	0	0	10	10
2 (low-dose)	2	20	10	10
3 (mid-dose)	20	75	10	10
4 (high dose)	75	250	10	10

Data taken from p. 20, MRID 45000509.

3. Dose selection rationale

Doses were based on two previous subchronic feeding studies. In the first study, male and female Fischer 344 rats were administered DE-537 NBU in the diet at concentrations designed to deliver 0, 3, 25, 100, or 400 mg/kg/day and 0, 10, 100, 400, or 800 mg/kg/day, respectively. Male body weights were not affected, but females administered 800 mg/kg/day had a 14% decrease in body weight and a corresponding reduction in food intake. Absolute and relative liver weights in males and females were increased in the three highest dose groups. Absolute and relative kidney weights were increased in males in the 400 mg/kg/day group and relative kidney weights were increased in females administered 800 mg/kg/day. Hepatocellular hypertrophy was observed in males at doses ≥25 mg/kg/day and in females administered ≥100 mg/kg/day.

In the second study, male and female Fischer 344 rats deficient in dipeptidyl peptidase IV were administered DE-537 NBU at fixed concentrations of 0, 30, 300, 1000, or 3000 ppm in the diet. Calculated doses for males were 0, 1.7, 17, 60, and 190 mg/kg/day and for females were 0, 2, 20, 65, and 200 mg/kg/day. Absolute and relative liver weights were significantly increased in both sexes administered 1000 and 3000 ppm and in male rats administered 300 ppm. Absolute kidney weights were significantly increased for males at ≥300 ppm and for females at 3000 ppm.

Relative kidney weights were increased for males at ≥1000 ppm and for females at 3000 ppm. At necropsy, darkened livers and kidneys were observed for all rats that received 3000 ppm and for some rats that received 1000 ppm. Microscopically, centrilobular hepatocellular swelling with minute eosinophilic granules was present in both sexes at 3000 ppm, in males at 1000 ppm, and in some males at 300 ppm. The proximal tubules of the kidneys of both sexes that received 3000 ppm contained deposits of lipofuscin. Males administered 1000 and 3000 ppm also had a slight anemia.

These studies demonstrated a three-fold greater effect in males than in females at comparable dose levels. High doses of 75 mg/kg/day for males and 250 mg/kg/day for females were chosen for the present study and were expected to result in liver enlargement and hepatocellular hypertrophy.

4. Test material preparation and analysis

Diets were prepared by adding an appropriate amount of the test material to a small amount of feed to establish a premix. These premixes were prepared approximately monthly in accordance with stability information. The premix was serially diluted with ground feed to achieve the lower concentrations. In order to maintain a constant dose, diets were prepared weekly from the premix based on the previous week's body weights and feed consumption. For homogeneity analysis, three samples were taken from both the top and bottom of the 2 and 250 mg/kg/day mixes on two different sampling times (first and second weeks of the study) and from the premix at the second sampling time. Stability for 6, 13, 32, and 123 days in rodent feed was established in an earlier dietary study (cited in MRID 45000412; Brzak, 1989). Details of the storage conditions were not provided. For concentration verification, diets were sampled at four time points. Samples from the control, premix, and all test diets were extracted and analyzed by HPLC separation with UV detection.

Results -

Homogeneity analysis: The concentrations (% w/w) of the six samples from the 2 and 250 mg/kg/day diets averaged 0.00227±0.00133 and 0.269±0.065, respectively. The relative standard deviations were 58.59 and 24.16%, respectively. As these standard deviations were unacceptable, the diets prepared during the second week were mixed using acetone to disperse the test material. Concentrations for the 2 and 250 mg/kg/day diets and premix prepared during the second week were 0.00293±0.00024, 0.313±0.007, and 1.01±0.01, respectively. The RSDs were 8.19, 2.24, and 0.99%. These RSDs were acceptable and diets during the following weeks were prepared using the new procedure.

Stability analysis: Stability data were provided in a previous report (MRID 45000412). In that study, the stability of DE-537 NBU in rodent feed following storage for 6, 13, and 32 days as determined by percent recovery was 96-102%. Following storage for 123 days, the percent recovery was 91%.

Concentration analysis: The mean percent of target concentrations averaged for the four sampling times were 2 mg/kg/day, 98.75; 20 mg/kg/day - males, 103.75; 20 mg/kg/day - females, 106.50; 75 mg/kg/day - males, 103.50; 75 mg/kg/day - females, 104.50; 250 mg/kg/day, 101.50; and premix, 100.50. Only one sample, 20 mg/kg/day - females during the fourth sampling, deviated by more than 13%.

The reported analytical data indicated that the mixing procedure was adequate (after the first week) and that the variance between targeted and actual dosage to the animals was acceptable.

5. Statistics

FOB incidence scores were analyzed with a z-test of proportions at $\alpha = 0.02$. Comparisons of 16 parameters between control and each treatment group, for each sex, at 5 time periods involved 480 z-tests. If more than one rank within the same observation had significant z scores, the toxicologically most significant rank was reported. Other FOB observations such as muscle tone were ranked (1-4 or 1-5) and averaged for each parameter at each observation time by dose level and sex. These scores were not quantitatively analyzed. For all continuous data, means and standard deviations were calculated and homogeneity of variance was evaluated with the Bartlett's test ($p = 0.01$). If the Bartlett's test was significant, the data were transformed. Body weight, rectal temperature, forelimb and hindlimb grip strength, landing foot splay, and motor activity were analyzed with a repeated-measures ANOVA with the factors of sex and treatment and the repeated factor of time. Motor activity (reported as the square root of the counts) also included subsessions. The multivariate index was the Pillai Trace statistic. The following interactions were evaluated: (1) treatment x time, (2) treatment x time x sex, and (3) treatment x time x subsession (motor activity only). For continuous data, the error rate was set at 0.05.

C. METHODS

1. Observations

All animals were observed cage-side twice each day for behavior and mortality. Clinical observations were performed weekly except weeks during which the FOB was conducted. Clinical examinations involved evaluations of the skin, fur, mucous membranes, respiration, nervous system function (tremors and convulsions), swelling, masses, and behavior.

2. Body weight

All animals were weighed pre-exposure and weekly during the study.

3. Food consumption and compound intake

Food consumption was determined weekly by weighing feeders at the start and end of a measurement cycle. Food consumption (g/day) was calculated as: (initial weight of feeder - final weight of feeder)/number of days in measurement cycle. Although diets were adjusted weekly to maintain a constant dose, actual compound intake was calculated by the study authors from data on weekly group mean body weights, food consumption and concentrations of the test material in the feed.

4. Functional observational battery (FOB)

The FOB was conducted in a clear plastic box under red light at approximately the same time of day. All animals/sex/group were subjected to a baseline FOB one week prior to treatment. The FOB was repeated during weeks 2, 4, 8, and 13. The following FOB observations were performed by the same trained technician who was blind to the treatment of the animals; except for categorical observations, these observations were ranked with rankings ranging from 1-4 or 1-5. Hand-held and open field observations were combined by the study authors. The duration of the open field observation without human intervention was 1 minute.

a. Hand-held and open field observations

Resistance to removal, palpebral closure, lacrimation, pupil size, pupil reactivity, degree of salivation, muscle tone, extensor thrust, reactivity to handling, activity (ambulatory and rearing), responsiveness to noise, responsiveness to touch, responsiveness to tail pinch, urination, defecation, and gait.

b. Sensorimotor and neuromuscular measurements

Hindlimb and forelimb grip strengths and landing foot splay.

c. Other

Body temperature.

During the 13th week of treatment an auditory screen involving auditory brainstem responses to sound frequencies of 4, 8, 16, and 30 kHz was conducted on 5 rats/sex in the control and high-dose groups. This test is for mid- and high-frequency hearing loss.

5. Motor activity

Motor activity was measured preexposure and during weeks 2, 4, 8, and 13 in a quiet, light-attenuated room. Motor activity was monitored for 48 minutes (six 8-minute sessions) in one of 24 circular chambers bisected by an infrared photobeam. Motor activity was measured as the number of beam interruptions that lasted more

than 100 milliseconds and followed an interval between beam breaks that was greater than 100 milliseconds (to discount activity such as tail flicking or head bobbing). Motor activity was monitored with a computerized system. Data were presented as square roots of total counts.

6. Sacrifice and pathology

After 13 weeks of treatment, five randomly selected rats/sex/dose were injected with heparin and anesthetized with methoxyflurane. Rats were perfused via the left ventricle with phosphate buffer containing sodium nitrite followed by a phosphate-buffered solution of 1.5% glutaraldehyde and 4% formaldehyde. Major organs and tissues were examined grossly and preserved. Tissues from the central nervous system (brain and spinal cord), and muscles were embedded in paraffin, sectioned approximately 6 μ thick, and stained with hematoxylin and eosin. Spinal nerve roots, dorsal root ganglia, and peripheral nerves were osmicated, embedded in plastic (epoxy resin), sectioned, and stained with toluidine blue. The checked (X) tissues from the control and high-dose groups were examined microscopically:

X	CENTRAL NERVOUS SYSTEM	X	PERIPHERAL NERVOUS SYSTEM
	BRAIN		**PERIPHERAL NERVES**
X	Olfactory bulb	X	Sciatic
X	Forebrain	X	Sural
X	Cerebrum (frontal, parietal, temporal, occipital)	X	Tibial with branches
	Midbrain		Peroneal
X	Cerebellum		**OTHER**
X	Pons		
X	Medulla oblongata	X	Lumbar dorsal root ganglion with roots
X	Thalamus/hypothalamus	X	Cervical dorsal root ganglion with roots
X	Pituitary	X	Anterior tibial muscle
X		X	Gastrocnemius muscle
	SPINAL CORD		
	Cervical swelling		
X	Lumbar swelling		
X	Thoracic swelling		
	OTHER		
	Gasserian ganglion		
X	Trigeminal nerves		
X	Optic nerves		
X	Eyes		
X	Olfactory epithelium		

The remaining animals that survived to term were sacrificed by carbon dioxide asphyxiation; additional tissues were not collected.

6. Positive and Historical Control Data

A statement addressing the ability of the technician to detect major neurotoxic endpoints was presented in Appendix C of the study report. Positive control substances included chlorpromazine, d-amphetamine sulfate, and atropine plus physostigmine sulfate; saline was the negative control substance. Head weaving and piloerection were observed in rats treated with d-amphetamine. Chlorpromazine-treated rats held fixed postures and atropine-physostigmine treated rats had tremors and a decreased response to tail pinch. The statement also addressed ability to measure body temperature, grip strength, and foot splay. No actual data were presented in Appendix C; the raw data are archived under report #T1.05-022-000-010.

Positive control data showing an increase and decrease in activity in rats treated with single doses of amphetamine and chlorpromazine, respectively, were presented in Appendix D of the study report. The historical data from control rats showed that habituation was reached during the 5th-6th 8-minute motor activity subsession. The study authors cite a Dow Chemical Company validation study that indicates that activity of control animals approaches asymptote in 30-40 minutes. The latter report was not provided to the reviewer.

Positive control data for neuropathology were presented in Appendix E of the study report. All findings were graded to reflect the severity of the specific lesions. Degeneration of smaller or more distal peripheral nerves was demonstrated in rats treated for three weeks with acrylamide in the drinking water. "The lesions were characterized by degeneration and loss of continuity of the axon with formation of myelin ovoids and the presence of phagocytic cells." Rats treated with a single dose of trimethyltin were observed to have lesions of the hippocampus and adjacent piriform cortex of the brain (not found in control or acrylamide-treated rats). Trimethyltin-treated rats were also observed to have a higher incidence than controls of individual nerve fiber degeneration in the cervical and lumbar spinal cord sections, peroneal nerve, and proximal sciatic nerve.

II. RESULTS

A. CLINICAL OBSERVATIONS AND MORTALITY

There were no deaths and no treatment-related effects were observed in any rats at any of the clinical observations times. Late in treatment, non-treatment-related periocular soiling was observed in a few animals; incidences were higher in the control group than in the treatment groups. One female in the 20 mg/kg/day group exhibited perineal urine soiling on several occasions.

B. BODY WEIGHT AND WEIGHT GAIN

Group mean body weights and total body weight gains are shown in Table 2. There was no effect of treatment on final body weights which ranged from 99 to 106% of the mean control weight for males and 98 to 102% of the mean control weight for females. Compared with the respective control group, body weight gains ranged from 98 to 109% for males and 98 to 104% for females.

TABLE 2. Group mean body weights (g) for selected weeks and total body weight gains (g) of rats administered DE-537 NBU in the diet for 13 weeks[a]

Males				
Week of study	Dose (mg/kg/day)			
	0	2	20	75
Pretreatment	123.6±7.1	124.8±7.9	124.1±7.4	124.4±5.7
2	183.9±12.6	191.9±9.7	189.4±9.5	186.5±8.3
4	227.9±11.1	241.5±9.0	233.7±11.5	229.4±8.2
8	279.2±11.6	296.5±9.5	284.0±18.3	277.1±12.6
13	311.4±14.2	328.7±12.5 (106)	314.2±22.1 (101)	309.0±15.0 (99)
Total weight gain[b]	188	204 (109)	190 (101)	185 (98)
Females				
Week of study	Dose (mg/kg)			
	0	20	75	250
Pretreatment	91.6±3.4	90.9±3.1	90.9±3.1	92.1±2.0
2	126.1±7.6	122.7±3.8	123.6±4.8	126.9±6.2
4	145.6±9.6	142.9±5.6	146.0±7.5	149.7±7.3
8	169.9±12.4	165.4±9.2	170.8±10.3	172.9±8.6
13	183.9±11.3	181.0±8.8 (98)	186.7±11.7 (102)	187.7±9.5 (102)
Total weight gain[b]	92	90 (98)	96 (104)	96 (104)

Data taken from Table 19, p. 114, MRID 45000509.
[a] Percent of control value in parenthesis; calculated by reviewer.
[b] Calculated by reviewer.

C. FOOD CONSUMPTION AND COMPOUND INTAKE

Weekly food consumption values did not differ among groups for either sex. Only one weekly mean value, food intake of males in the low-dose group during the 3rd week of the study, was significantly increased ($p = 0.05$) compared with the control group mean.

Test material intake was calculated by the study authors based on mean food intakes and body weights. Actual test material intakes for males in the low, mid, and high dose groups were 2.1, 21.0, and 78.9 mg/kg/day, respectively. Respective intakes for females were 20.6, 77.1, and 257.1 mg/kg/day.

D. FUNCTIONAL OBSERVATIONAL BATTERY

Because of the large number of pair-wise statistical comparisons of the ranked data (5 time periods, 16 observations, 2 sexes, and 3 control vs treatment comparisons), there were several false positive findings (ranked differences of 0.5 flagged) among the 480 pair-wise comparisons. For example, three comparisons were flagged preexposure: resistance of males in the 75 mg/kg/day group to removal from the cage and urination in mid-dose males and high-dose females. During treatment, ranked comparisons of 0.5 or greater between control and treatment groups were randomly distributed among time periods, sexes, and exposure groups. Even when the high-dose group of either sex was flagged, the data failed to show a dose-response relationship among the treatment groups. Discounting the four significance differences in paired rankings pretreatment ($\alpha = 0.02$), only urination in mid-dose males during week 13 of treatment attained significance. This effect (lack of urination in 9 males in the treated group vs 4 in the control group) was clearly not dose-related. It should be noted that in all cases, behavior and gait did not differ among groups and was considered within normal limits. There were no treatment-related effects for either sex at any time in forelimb and hindlimb grip strength, landing foot splay, or rectal temperature.

The auditory screen failed to reveal an effect of treatment on hearing. Composite auditory brainstem response waveforms for males and females in the control and high-dose groups were similar at all four auditory stimulus frequencies.

D. MOTOR ACTIVITY

Motor activities for the total 48-minute sessions during weeks 2, 4, 8, and 13 were not affected by treatment at any time for either sex (Table 3), i.e., there was no variation between treatment x day, treatment x day x sex, or treatment x day x subsession. Motor activity decreased across the six subsessions for all groups preexposure and for the six subsessions of the male and female control group during different weeks. The data showed that habituation was reached by the 5^{th} to 6^{th} subsession of the 48-minute period.

TABLE 3. Motor activity of rats administered DE-537 NBU in the diet for 13 weeks[a]				
Males				
Week of study	**Dose (mg/kg/day)**			
	0	**2**	**20**	**75**
Pretreatment	9.78±1.36	10.43±2.60	9.33±1.97	10.26±2.66
2	10.53±2.18	10.72±1.88	10.76±2.04	10.78±1.77
4	11.12±1.25	10.87±1.69	10.80±0.98	11.55±1.41
8	11.01±1.87	10.21±1.14	11.16±0.57	11.61±1.51
13	10.76±1.49	10.38±1.70	10.06±1.72	10.03±0.88
Females				
Week of study	**Dose (mg/kg/day)**			
	0	**20**	**75**	**250**
Pretreatment	10.30±2.14	10.36±1.90	9.70±1.23	9.22±1.11
2	12.82±1.87	12.78±1.43	13.36±2.20	12.15±2.36
4	12.86±1.89	13.21±2.65	13.21±1.72	14.12±1.91
8	13.40±1.93	13.14±1.18	12.84±2.01	13.51±1.59
13	12.55±1.68	13.59±1.53	12.61±1.40	12.27±1.70

Data taken from Table 31, p. 128, MRID 45000509.
[a]Values are square roots of total counts.

E. SACRIFICE AND PATHOLOGY

1. Organ weight

Organs were not weighed as part of this neurotoxicity study.

2. Gross pathology

Livers were grossly increased in size in 5/5 males and 4/5 females in the respective high-dose groups.

3. Neuropathology

No dose-related or treatment-related lesions of the central or peripheral nervous system were observed. Very slight degeneration of individual nerve fibers of several structures including the trapezoid body of the medulla oblongata, spinal cord, and peripheral nerves as well as atrophy of the optic nerve and mineralization of optic arteries and the cornea of the eye were observed. Incidences were low and were scattered among different nerves and dose groups including the control group.

III. DISCUSSION

A. DISCUSSION

Administration of DE-537 NBU to male and female Fischer 344 rats in the diet at target doses of 0, 2, 20, or 75 mg/kg/day (males) or 0, 20, 75, or 250 mg/kg/day (females) for 13 weeks resulted in no treatment-related clinical signs and had no effect on body weight, food intake, behavior (as determined by FOB and motor activity evaluations), or the central or peripheral nervous system (as determined by neuropathologic evaluations). Livers in the male and female high-dose groups, observed grossly, were characterized as enlarged. OPPTS Guideline 870.6200 does not call for organ weights. Therefore, the enlarged livers were not considered a toxicological endpoint in this study. The measured test material intake was close to and exceeded the target doses. Therefore, no correction for actual intake needs to be made. The statistical evaluation procedure chosen by the study authors resulted in a number of false positive findings in the FOB (example, amount of urination in some treatment groups both preexposure and during treatment), but the lack of any dose-response relationship made it clear that these positive findings were not treatment related.

The choice of doses in this study provides a dilemma as higher doses obviously could have been administered. The authors based doses in the present study on previous subchronic studies in which liver hypertrophy (without accompanying lesions) was evident. At the time the previous subchronic studies were performed and the present study initiated, liver hypertrophy was considered an adverse effect. Liver hypertrophy alone is no longer considered an adverse effect and higher doses should have been administered in order to attempt to attain a LOAEL for neurotoxicity. Because the authors made a good faith effort to follow guidelines in place at the time, the study should not be rejected for failure to attain a LOAEL.

This study closely followed OPPTS 870.6200 guidelines. The appropriate tests were described and performed and the results were properly reported. Historical and positive control data and the ability of the technician to detect behavioral effects and of the pathologist to detect neuropathologic effects were demonstrated. Not all of the positive and historical control information was dated, but most of it was performed within the last 10 years. Although the positive and historical control data were not collected concurrent with the present study, the data presented, including published journal articles, clearly demonstrates the ability of the testing laboratory to detect neurotoxic endpoints.

Based on the lack of effects seen in this study, the NOAEL for neurotoxicity of DE-537 NBU was ≥75 mg/kg/day in male rats and ≥250 mg/kg/day in female rats. LOAELs could not be identified.

B. STUDY DEFICIENCIES

The raw data demonstrating the ability of the technician to detect neurologic endpoints in the FOB were not provided. However, a statement of this ability was provided in Appendix C the study report. In addition, positive control data provided in an accompanying acute neurotoxicity study (MRID 45000409) provide evidence that technicians at The Dow Chemical Company Health & Environmental Research Laboratories are proficient in conducting the FOB.

DATA EVALUATION RECORD

[Corrected Cover Page]

CYHALOFOP BUTYL
(XDE-537 BE)

STUDY TYPE: GENERAL PHARMACOLOGY–SPECIAL STUDY IN MICE AND RABBITS

[OPPTS: 870.xxxx]
MRID 45000424

Prepared for

Office of Pesticide Programs
U.S. Environmental Protection Agency
1921 Jefferson Davis Highway
Arlington, VA 22202

Prepared by

Chemical Hazard Evaluation Group
Toxicology and Risk Analysis Section
Life Sciences Division
Oak Ridge National Laboratory
Oak Ridge, TN 37831
Task Order No. 01-81-HH

Primary Reviewer:		
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Robert H. Ross, M.S., Group Leader	Signature:	_____
	Date:	_____
Quality Assurance:		
Lee Ann Wilson, M.A.	Signature:	_____
	Date:	_____

Disclaimer

This review may have been altered subsequent to the contractor's signatures above.

Oak Ridge National Laboratory, managed by UTBattelle, LLC, for the U.S. Dept. of Energy under contract DEAC0500OR22725

DATA EVALUATION RECORD

CYHALOFOP BUTYL
(XDE-537 BE)

STUDY TYPE: ~~METABOLISM AND PHARMACOKINETICS~~ (SPECIAL STUDY)
~~[OPPTS: 870.7485 (§85-1)]~~
MRID 45000424

Prepared for

Office of Pesticide Programs
U.S. Environmental Protection Agency
1921 Jefferson Davis Highway
Arlington, VA 22202

Prepared by

Chemical Hazard Evaluation Group
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Task Order No. 01-81-HH

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Disclaimer

This review may have been altered subsequent to the contractor's signatures above.

Oak Ridge National Laboratory, managed by UTBattelle, LLC, for the U.S. Dept. of Energy under contract DEAC0500OR22725

CYHALOFOP BUTYL (XDE-537 BE) **Special Study [OPPTS 870.xxxx]**

EPA Reviewer: John Whalan, Date 5-24-01
Registration Action Branch 2 (7509C)
EPA Work Assignment Manager: SanYvette Williams-Foy, D.V.M, Date 5/21/01
Registration Action Branch 2 (7509C)
TXR No: 0050348

DATA EVALUATION RECORD

STUDY TYPE: General Pharmacology [OPPTS 870.xxxx] Special Study in Mice and Rabbits

DP BARCODE: D268553 SUBMISSION CODE: S
P.C. CODE: 082583 TOX. CHEM. NO.: Not available

TEST MATERIAL (PURITY): XRD-537 BE (97.1%)

SYNONYMS: R-(+)-n-butyl-2-[4-(2-fluoro-4-cyanophenoxy)phenyl]propanoate; XDE-537; XRD-537; XRD-537 n Bu; XDE-537 n Bu; XRD-537 n butyl ester; DEH-112; cyhalofop butyl

CITATION: 1) Takahashi, H. (1992). XDE-537 BE: General pharmacological study. Toxicology Division, Mitsukaido Laboratories, The Institute of Environmental Toxicology, 4321 Uchimoriya-cho, Mitsukaido-shi, Ibaraki, 303, Japan. Laboratory Study ID IET 91-00118. MRID 45000424. August 25, 1992. Unpublished.

SPONSOR: DowElanco Japan, Ltd., Seavans North, 21-, Shibaura 1-chome, Minato-ku, Tokyo 105 Japan.
Nichimen Corporation, 11-1, Nihonbashi 3-chome, Chuo-ku, Tokyo 103, Japan.

EXECUTIVE SUMMARY: In a special study (MRID 45000424), groups of three male and three female white SPF mice were given a single intraperitoneal dose (0, 4.88, 19.5, 78.1, 313, 1250, or 5000 mg/kg) of XRD-537 BE (Lot No. AGR-295713, chemical purity 97.1%). Additionally, groups of three to six male white rabbits were given a single oral dose (0, 313, 1250, 2500, or 5000 mg/kg) of XRD-537 BE. Behavioral and neuromuscular responses (spontaneous activity, body posture, response to noise, muscle tone, various reflexes, motor coordination), and autonomic responses (pupil size, palpebral reflex, respiration, body temperature, heart rate, gastrointestinal activity) were monitored up to one week in both mice and rabbits. Additionally, cardiopulmonary parameters (respiratory rate, heart rate, blood pressure, and EKG) were assessed in three high-dose rabbits.

A 100% mortality was observed for both male and female mice within 24 hours following a single intraperitoneal dose of 1250 or 5000 mg/kg. Death occurred as early as three hours in the highest dose group and was preceded by behavioral and motor function abnormalities (e.g., alterations in alertness, visual placing, spontaneous activity, motor incoordination, decreased muscle tone, and compromised autonomic reflexes), some of which appeared as early as 30 minutes postdosing. Male and female mice responded similarly. At 313 mg/kg, only minimal effects were observed in the mice including decreased spontaneous activity, minor alterations in

muscle tone and minor changes in autonomic functions (slight hyperthermia, slightly decreased respiratory rate).

For rabbits, one of three animals in the 5000 mg/kg dose group exhibited decreased spontaneous activity, prostration, decreased muscle tone, compromised autonomic reflexes, and decreased respiratory and heart rate at one day after dosing. This rabbit died on Day 4. There were no clinically significant findings in the remaining rabbits of the 5000 mg/kg dose group or any lower dose groups. Additionally, there were no significant effects on EKGs or blood pressure in any of the rabbits given the test article.

Mice (single I.P. dose):

NOAEL = 78.1 mg/kg

LOAEL = 313 mg/kg (based on minimal effects including decreased spontaneous activity, minor alterations in muscle tone and minor changes in autonomic functions such as slight hyperthermia, and slightly decreased respiratory rate).

LD = 1250 mg/kg

Rabbits (single oral dose):

NOAEL - 2500 mg/kg

LOAEL = 5000 mg/kg (based on the response of one of three test subjects including decreased spontaneous activity, prostration, decreased muscle tone, compromised autonomic reflexes, decreased respiratory and heart rate at one day after dosing, and death on day 4)

This special study in mice and rabbits (submitted under §85-1 Guidelines) is **Acceptable/ NonGuideline**. Although the study was properly conducted and data were provided regarding the pharmacologic/toxicologic effects in mice following a single intraperitoneal dose up to 5000 mg/kg and in rabbits following a single oral dose up to 5000 mg/kg, the study does not meet criteria for any Guideline studies. It may be considered as a preliminary range-finding study for the routes of administration examined. It also provides insight into the pharmacologic/ toxicologic properties of the test article.

COMPLIANCE: Signed and dated Good Laboratory Practice Compliance, Quality Assurance, and Data Confidentiality statements were included with the studies.

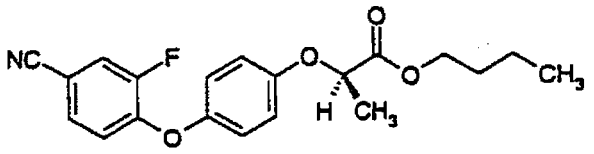
I. MATERIALS AND METHODS

A. MATERIALS

1. Test compound

Test article: XRD-537 BE
Lot No.: AGR-295713
Chemical purity: 97.1%
CAS No.: 122008-85-9
Description: not provided
Contaminants: no data provided

Structure:



2. Vehicle

The test article was not readily soluble in water and, therefore, was suspended in 1% Tween 80.

3. Test animals

Species: male and female mice; male albino rabbits
Strain: ICR SPF mice (Charles River Japan, Inc., Kanagawa, Japan); Japanese white, SPF (Kitayama Labes Co., Ltd., Kyoto, Japan)
Age and weight at study initiation: mice: 7 weeks (males 33.9-40.1 g; females 24.3-30.9 g); rabbits: 10 weeks (2.4-2.9 Kg)
Housing: 3 mice/aluminum cage; rabbits kept individually in aluminum cages
Diet: mice were fed pelleted diet MF-1 (Oriental Yeast Co., Ltd. Tokyo, Japan) *ad libitum*; rabbits were fed certified pelleted diet RC4 (Oriental Yeast Co., Ltd. Tokyo, Japan) *ad libitum*
Water: filtered tapwater *ad libitum*
Environmental conditions:
Temperature:22±1 °C
Humidity: 55±10%
Air changes: 15/hr
Photoperiod: 12 hrs/12 hrs
Acclimation period: at least 1 week

4. Preparation of dosing solution

Test material was suspended

Results –
Homogeneity: The homogeneity of the dosing suspension could be assumed from its being uniformly suspended in Tween 80. However, no confirmatory data were provided.

Stability: No data were provided regarding the stability of the test article or the dosing suspensions.

Dose confirmation: No data were provided regarding dose confirmation.

B. STUDY DESIGN AND METHODS

1. Group arrangements

The experimental groups were established as shown in Table 1. All test animals were individually identified. Test animals were observed prior to dosing and clinical observations made at 0.5, 1, 3, and 6 hours after administration and once per day for one week. Observations entailed numeric grading of "normally present or absent signs"; extremely supranormal, supranormal, normal, subnormal, and extremely subnormal.

TABLE 1. Study Design for Evaluating Toxicologic Potential of XDE-537 BE (Cyhalofop) in Rats and Rabbits

Experiment group	Dose (mg/kg)	Number/Sex	Remarks
Mice			
1	0	3 Male/3 Female	Clinical observations up to 1 week
2	4.88 I.P.	3 Male/3 Female	Clinical observations up to 1 week
3	19.5 I.P.	3 Male/3 Female	Clinical observations up to 1 week
4	78.1 I.P.	3 Male/3 female	Clinical observations up to 1 week
5	313 I.P.	3 Male/3 Female	Clinical observations up to 1 week
6	1250 I.P.	3 Male/3 Female	Clinical observations up to 1 week
7	5000 I.P.	3 Male/3 Female	Clinical observations up to 1 week
Rabbits			
8	0	6 Males	Clinical observations up to 1 week; 3 rabbits for cardiopulmonary evaluation
9	313 oral	3 Males	Clinical observations up to 1 week
10	1250 oral	3 Males	Clinical observations up to 1 week
11	2500 oral	3 Males	Clinical observations up to 1 week
12	5000 oral	6 Males	Clinical observations up to 1 week; 3 rabbits for cardiopulmonary evaluation

Information taken from p 4, MRID 45000424.

2. Dosing and sample collection

A single dose was given intraperitoneally (I.P.) to mice and orally to rabbits (test suspension concentrations were adjusted to achieve a dose volume of 20 mL/kg). Control groups received equivalent volumes of the 1% Tween 80 vehicle without the test article. The I.P. administration represented a total absorption situation and the oral route represented the most likely route responsible for acute poisoning.

Samples of expired air, urine, feces, cage, washes, blood, and tissues normally collected for 85-1 Guideline studies were not collected.

3. Sample preparation/analysis

Analytical procedures as outlined for 85-1 Guideline studies were not conducted.

4. Clinical observations

Mice - The following categories of behaviors/responses were monitored: awareness, mood, motor activity, CNS excitation, posture, motor incoordination, muscle tone, autonomic and skeletal muscle reflexes. No details were provided regarding the procedures for these assessments.

Rabbits - A wide array of behavioral and neuromuscular responses were monitored including spontaneous activity, body posture, response to noise, muscle tone, various reflexes, motor coordination, and autonomic responses (pupil size, palpebral reflex, respiration, body temperature, heart rate, gastrointestinal activity. No details were provided regarding the assessment of these parameters.

5. Cardiopulmonary evaluations

Effects of the test article on respiration, blood pressure, and cardiac electrical activity in unanesthetized rabbits were assessed at 0.5, 1, 3, 6, and 24 hours after a single 5000 mg/kg oral dose of the test article. Three male rabbits per dose group were used including three rabbits serving as untreated controls. Electrocardiograms (EKG) were recorded on a polygraph (RM-6000, Niohon Kohden, Inc., Tokyo, Japan). Respiratory parameters were assessed with a thermistor inserted into the nostrils and a digital counter. Blood pressure and heart rate were measured using a cannula in the femoral artery (previously inserted while the rabbit was anesthetized) and a high-pressure transducer. The apparatus was flushed with saline to prevent coagulation.

6. Calculations and Statistics

Results from test groups were compared with respective control groups using Student's t-test with significance determined at $p \leq 0.05$.

II. RESULTS

Results of this study were limited to findings pertaining to the clinical and cardiopulmonary observations previously summarized in I.B.4 and I.B.5. No data (e.g., mass balance, absorption, distribution, excretion, metabolite profiles) typical of an 85-1 Guideline study were evaluated in this special study

A. GENERAL TOXICOLOGIC EFFECTS

1. Clinical observations

Mice - All male and female mice in the 1250 mg/kg and 5000 mg/kg I.P. dose groups died within one day after dosing. Deaths began occurring at 6 hours after dosing in the 1250 mg/kg group and at 3 hours after dosing in the 5000 mg/kg group. Death was preceded by notable motor and behavioral abnormalities, most of which began within 30 minutes to one hour after dosing. These effects included alterations in alertness, visual placing, spontaneous activity, motor incoordination, decreased muscle tone, and compromised autonomic reflexes. There did not appear to be notable differences in responses of male and female mice. At doses of 313 mg/kg, only minimal effects were observed in the mice including decreased spontaneous activity, minor alterations in muscle tone and minor changes in autonomic functions (slight hyperthermia, slightly decreased respiratory rate). No clinically significant abnormalities were observed in dose groups receiving 78.1 mg/kg or less.

Rabbits - One male rabbit of three receiving a single oral dose of 5000 mg/kg died on Day 4. The rabbit exhibited decreased motor activity, decreased muscle tone and reflexes, and decreased heart and respiratory rates over the three days prior to its death. No other significant clinical findings were observed for the remaining rabbits in the high dose group or any rabbits in the lower dose groups.

2. Cardiopulmonary evaluations

There were no clinically significant effects on the assessed cardiopulmonary parameters (i.e., respiratory rate, EKG, heart rate, blood pressure) in the rabbits examined.

B. PHARMACOKINETIC STUDIES

Pharmacokinetic studies were not a protocol element of this special study.

C. METABOLITE CHARACTERIZATION STUDIES

Metabolite characterization studies were not a protocol element of this special study.

III. DISCUSSION

A. DISCUSSION

In a special study (MRID 45000424), groups of three male and three female white SPF mice were given a single I.P. dose (0, 4.88, 19.5, 78.1, 313, 1250, or 5000 mg/kg) of XRD-537 BE (Lot No. AGR-295713, chemical purity 97.1%). Additionally, groups of three to six male white rabbits were given a single oral dose (0, 313, 1250, 2500, 5000

mg/kg) of XRD-537 BE. Behavioral and neuromuscular responses (spontaneous activity, body posture, response to noise, muscle tone, various reflexes, motor coordination), and autonomic responses (pupil size, palpebral reflex, respiration, body temperature, heart rate, gastrointestinal activity) were monitored up to one week in both mice and rabbits. Additionally, cardiopulmonary parameters (respiratory rate, heart rate, blood pressure, and EKG) were assessed in three high-dose rabbits.

A 100% mortality was observed for both male and female mice within 24 hours following a single intraperitoneal dose of 1250 or 5000 mg/kg. Death occurred as early as three hours in the highest dose group and was preceded by behavioral and motor function abnormalities (e.g., alterations in alertness, visual placing, spontaneous activity, motor incoordination, decreased muscle tone, and compromised autonomic reflexes), some of which appeared as early as 30 minutes postdosing. Male and female mice responded similarly. At doses of 313 mg/kg, only minimal effects were observed in the mice including decreased spontaneous activity, minor alterations in muscle tone and minor changes in autonomic functions (slight hyperthermia, slightly decreased respiratory rate).

For rabbits, one of three animals in the 5000 mg/kg dose group exhibited decreased spontaneous activity, prostration, decreased muscle tone, compromised autonomic reflexes, and decreased respiratory and heart rate at one day after dosing. This rabbit died on Day 4. There were no clinically significant findings in the remaining rabbits of the 5000 mg/kg dose group or any lower dose groups. Additionally, there were no significant effects on EKGs or blood pressure in any of the rabbits given the test article.

This special study served to provide preliminary findings regarding possible pharmacologic/toxicologic properties of the test article in mice following a single I.P. administration and in rabbits following a single oral dose. **Based on neuromuscular and behavioral effects in male and female mice a NOAEL of 78.1 mg/kg and LOAEL of 313 mg/kg could be estimated for a single I.P. dose. At doses of 1250 and 5000 mg/kg, 100% mortality occurred. A LOAEL of 5000 mg/kg could be estimated for rabbits based on the response of one of three test subjects. Based on the absence of neuromuscular, cardiac and respiratory effects in male rabbits, a NOAEL of 2500 mg/kg could be estimated for single oral exposure.**

This special study in mice and rabbits (submitted under §85-1 Guidelines) is **Acceptable/ NonGuideline**. Although the study was properly conducted and data were provided regarding the pharmacologic/toxicologic effects in mice following a single I.P. dose up to 5000 mg/kg and in rabbits following a single oral dose up to 5000 mg/kg, the study does not meet criteria for any Guideline studies but may be considered as a preliminary range-finding study for the routes of administration examined.

B. STUDY DEFICIENCIES

Although submitted as an §85-1 study, the study protocol was not that of a metabolism/disposition study and, therefore, did not satisfy any §85-1 Guideline requirements. The study appeared to be properly conducted and the data adequately were reported although protocol details (e.g. methods for evaluating the various behavioral and neuromotor responses/reflexes) were lacking.

DATA EVALUATION RECORD

CYHALOFOP BUTYL
(XRD-537 BE)

STUDY TYPE: METABOLISM AND PHARMACOKINETICS – DOG
[OPPTS: 870.7485 (§85-1)]
MRID 45000425

Prepared for

Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
1921 Jefferson Davis Highway
Arlington, VA 22202

Prepared by

Chemical Hazard Evaluation Group
Toxicology and Risk Analysis Section
Life Sciences Division
Oak Ridge National Laboratory
Oak Ridge, TN 37831
Task Order No. 01-81-II

Primary Reviewer: Robert A. Young, Ph.D., D.A.B.T.	Signature: Date:	Robert A. Young FEB 0 9 2001
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Disclaimer

This review may have been altered subsequent to the contractor's signatures above.

Managed and operated by UT-Batelle, LLC, for the U.S. Department of Energy under Contract No. DE-AC05-00OR227525.

CYHALOFOP BUTYL (XRD-537 BE) **Metabolism Study [OPPTS 870.7485 (§85-1)]**

EPA Reviewer: John Whalan, Date 5-4-01
Registration Action Branch 2 (7509C)
EPA Work Assignment Manager: SanYvette Williams-Foy, D.V.M., Date 5/31/01
Registration Action Branch 2 (7509C)
TXR No: 0050348

DATA EVALUATION RECORD

STUDY TYPE: Metabolism - Dog [OPPTS 870.7485 (§85-1)]

DP BARCODE: D268553
P.C. CODE: 082583

SUBMISSION CODE: S
TOX. CHEM. NO.: NA

TEST MATERIAL (PURITY): XRD-537 BE (>97%)

SYNONYMS: R-(+)-n-butyl-2-[4-(2-fluoro-4-cyanophenoxy)phenyl]propanoate; XDE-537; XRD-537; XRD-537 n Bu; XDE-537 n Bu; XRD-537 n butyl ester; DEH-112; cyhalofop butyl

CITATION: Ninomiya, S-i., et al. (1995). XRD-537 BE: Absorption , metabolism and excretion preliminary study in the dog. Tokai Research Laboratories, Daiichi Pure Chemicals Co. Ltd., 2117 Muramata, Tokai-mura, Naka-gun, Ibaraki 319-11, Japan. Laboratory Study ID GHF-R-295. MRID 45000425. March 3, 1995. Unpublished.

SPONSOR: Dow Chemical Japan Limited; Nichimen Corporation.

EXECUTIVE SUMMARY: In a metabolism and disposition study (MRID 45000425) two male beagle dogs were gavaged with [α-^{14}C]XRD-537 BE (>97% radiochemical purity, Lot no. A-903-48, 2.01 MBq/mg) and nonlabeled XRD-537 (97% chemical purity, Lot no. AGR 295713) at a dose of 1 mg/kg. Blood, urine, and feces were collected up to 168 hours after dosing. Metabolite profiles and metabolite characterizations were performed for all three matrices.

No treatment-related adverse effects were reported for either of the two dogs. Overall recovery of administered radioactivity was an acceptable 93.8 and 95.2% for each of two dogs. Based upon urinary excretion and blood time-course data, approximately 50% of a single oral dose of the test article was absorbed with most absorption occurring over several hours. The absence of biliary excretion data prevented reliable evaluation of total absorption. Both the urine and feces were major routes of elimination and were quantitatively similar. Urinary excretion accounted for 42.5 and 43.9% of the administered dose over 168 hours and was 90% complete within 72 hours. Over 168 hours, fecal excretion accounted for 50.6 and 48.6% of the administered radioactivity in each of two dogs and was also 85-90% complete within 72 hours. There were no data on tissue distribution of administered radioactivity.

Time-course analysis of blood/plasma radioactivity showed maximum concentrations at 1-2 hours for both blood (1.78 - 1.87 μg eq./mL) and plasma (2.95 - 2.97 μg eq./mL). Clearance from plasma and blood was not especially rapid but nearly complete after 48 hours. The limited data from only two dogs suggested a biphasic clearance with a slight plateau or spike at

approximately 24-36 hours. There was measurable radioactivity in both matrices at 168 hours. Area-under-the-curve (AUC) was approximately 1.75-fold greater for plasma than for whole blood, possibly indicative of protein binding.

This study provided data showing that at least 50% of a single oral dose (1 mg/kg) of XRD-537 BE to dogs was absorbed over a 168-hour period and that both the feces and urine were major routes of excretion. The test article appears to be metabolized primarily by hydrolysis to R-(+)-2-[4-cyano-2-fluorophenoxy)phenoxy]propanoic acid which was found in both the urine and feces. Several other metabolites were also formed, each representing <5% of the administered dose. No parent compound was found in the urine, and only minimal amounts were detected in the feces.

This metabolism study in rats is **Acceptable/Non-guideline**. The study used only two male dogs rather than four and did not provide data regarding tissue distribution. Therefore, it does not meet the minimum requirements for a Tier 1 metabolism study. Although not satisfying the requirements for a Metabolism and Pharmacokinetics Study [OPPTS 870.7485 (85-1)], this study (MRID 45000425) was properly conducted and reported, and provided useful data regarding the absorption, metabolism and excretion of XRD-537 BE in dogs following a single oral dose.

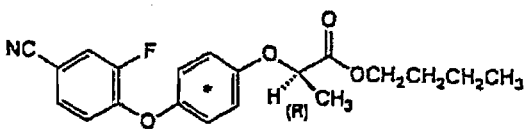
COMPLIANCE: Good Laboratory Practice, Quality Assurance, and Data Confidentiality statements were included with the study report. Flagging statements were not included.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test compound

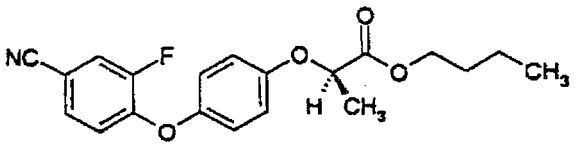
Radiolabeled: [∝-^{14}C]XRD-537 BE
Lot No.: A-903-48
Specific Activity: 2.01 MBq/mg
Radiochemical purity: >97%
Description: not provided
Contaminants: none noted
Structure:



* : ^{14}C

Non-radiolabeled: XRD-537 BE
Lot No.: AGR 295713
Purity: 97%
Description:
CAS No.: not provided

Structure:



2. Vehicle

Distilled water for injection (Hikari Pharmaceutical Co., Ltd.; Lot No. 9008ST), carboxymethyl cellulose sodium (Wako Pure Chemical Industries, Ltd.; CMC, Lot No. WDP1183), and Tween 80 (Wako Pure Chemical Industries, Ltd., CMC; Lot NO. TWP5590).

3. Test animals

Species: dog (male); Hazelton-LRE, 6321 South 6th Street, Kalamazoo, MI.
Strain: beagle
Age and weight at study: 6-8 months; 8.6 and 10.3 kg
Housing: housed individually in stainless steel cages (70 cm x 70 cm x 70 cm)
Diet: diet for dogs (DS-5; Oriental Yeast Co., Ltd.) provided *ad libitum*
Water: water was provided *ad libitum*
Environmental conditions:
Temperature: 23 ±2°C
Humidity: 55±15 %
Air changes: at least 15/hr
Photoperiod: 12 hrs/12 hrs
Acclimation period: 40-55 days

4. Preparation of dosing solution

The dose solutions were formulated using appropriate amounts of labeled and nonlabeled test material in 0.5% carboxymethyl cellulose and Tween 80 to obtain a test article concentration of 2%. To assure homogeneity, the mixture was sonicated for ~30 minutes and stirred for ~15 minutes. The final dose suspension was 0.5 mg/mL which was stored at 4°C in the dark until used.

Results –
Homogeneity: The homogeneity of the dosing formulation was confirmed in a separate study (DPC/AE-1528-1G, MRID 45000528). (unavailable for review)

Stability: Stability of the dosing suspension was confirmed (DPC/AE-1528-1G, MRID 45000528) by no loss of radiochemical purity over 21 days. (unavailable for review)

Dose confirmation: Dose formulation was confirmed in a separate study (DPC/AE-1528-1G, MRID 45000528). (unavailable for review)

B. STUDY DESIGN AND METHODS

1. Group arrangements

Only one experimental group containing two male dogs was used in this preliminary study.

TABLE 1. Study design for Disposition and Metabolism of [α-^{14}C]XRD-537 BE in Dogs

Experiment group	Dose (mg/kg)	Number/Sex	Remarks
A	1	2 males	absorption and excretion (urine/feces); blood time-course and kinetics; metabolite identification

Information taken from p. 29, MRID 45000425.

2. Dosing and sample collection

The test material was administered orally via a catheter to two male nonfasting dogs. The dose was 1 mg/2 mL/kg with dose volume being measured with a gas-tight Hamilton syringe. Administered radioactivity was ~1.85 MBq/kg.

Expired air - Expired air was not collected.

Blood - Approximately 2 mL of blood were collected from the cephalic or saphenous vein into vacuum tubes containing heparin sodium. Collections were made at 15 and 30 minutes, and at 1, 2, 3, 4, 6, 8, 12, 24, 48, 96, 120, and 168 hours.

Urine - Urine samples were collected in iced-cooled containers at 4, 8, 12, and at 24-hour intervals thereafter up to 168 hours.

Feces - Feces were collected in iced-cooled containers at 4, 8, 12, and at 24-hour intervals thereafter up to 168 hours.

Cage wash - Cages were rinsed with 800 mL of distilled water at the end of each collection period.

Tissues - No additional tissue samples were taken.

3. Sample preparation/analysis

Blood - A 100 μL aliquot of whole blood was taken from each sample; the remainder of each sample was centrifuged to obtain plasma. The 100 μL aliquot of whole blood and a 100 μL aliquot of plasma were each added to 2 mL Soluene-350 (Packard). The whole blood sample was decolorized with benzoyl peroxide in benzene and all samples mixed with 13 mL Econofluor scintillant and analyzed for radioactivity. For metabolite analysis, plasma was added to acetate buffer and subjected to solid-phase extraction using a Bond Elute C_{18} Cartridge (Varian SPP) and a WP® C_4 cartridge.

Eluate was then analyzed for radioactivity. Liquid-phase extraction was also used for analysis of plasma samples. Lyophilized samples were mixed with methanol, sonicated and centrifuged. Extraction of the pellet was then repeated twice and aliquots of pooled supernatants diluted and analyzed for radioactivity. Residues were dissolved in Soluene-350, diluted with toluene, mixed with Hionicfluor and analyzed for radioactivity. The liquid-phase extraction provided better recovery of radioactivity from plasma samples (99% vs 81%) and, therefore, was the selected method for these samples.

Urine - Urine samples were diluted with distilled water to a volume of 500 mL. Aliquots (1 mL) were mixed with 10 mL of Atomlight (DuPont) scintillation fluid and analyzed for radioactivity. Urine samples were also subjected to solid-phase extraction as described for plasma samples.

Feces - Distilled water was added to each fecal sample to a final volume of 1500 mL and the mixtures homogenized. A 500-mL aliquot of the homogenate was diluted with distilled water to 1 mL and further homogenized. Soluene-350 (2 mL) and Econofluor (13 mL) were added to 1 mL aliquots of the final fecal homogenate and the preparation analyzed for radioactivity. Urine samples were also subjected to solid-phase extraction as described for plasma samples.

Cage wash - Cage wash samples were added to distilled water for a final volume of 1 L. The mixture was homogenized and 10 mL of Atomlight scintillant added to 1 mL aliquots of the homogenate.

4. Analytical techniques

Liquid Scintillation Counting (LSC) - Radioactivity was determined using LSC-903 (Aloka) or 2500TR (Packard) liquid scintillation counters. The cross channel ratio method and an external standard were used for assessing counting efficiency. Single aliquots of HPLC eluate, blood, and plasma samples were counted while other samples were counted in duplicate. Samples varying by more than 10% from one another were re-evaluated. Background was determined based upon sample from control animals.

High Performance Liquid Chromatography (HPLC) - HPLC analysis was performed using a Shimadzu Co., Ltd. LC-6A with a SCL-A controller, UV detector, and fraction collector. Separation was conducted using a Novapak C_{18} column with a guard column, a mobile phase of 0.5% acetic acid/acetonitrile and 0.5% acetic acid in water, and a flow rate of 1 mL/min. Detection was at 254 nm. Eluate was collected at 30-minute intervals.

Thin-Layer Chromatography (TLC)- Silica gel 60 F_{264} glass plates were used and developed (single or two-dimensional) to 15 cm. Both acidic and basic solvent systems were utilized. The specific solvent systems used for analyzing standards and metabolite fractions were adequately described in the study report. The developed plates were placed in contact with Type BA (Fuji Film) imaging plates and exposed

for 24 hours. Autoradiograms were prepared with a bio-image analyzer (Fujix BA100, Fuji Film).

Metabolite identification- Reference standards were available for characterization of the metabolite fractions obtained from analysis of plasma, urine, and feces. Enzymatic hydrolysis (β-glucuronidase and arylsulfatase) was used for identification of fractions as conjugation products. Both TLC and HPLC methods were used to obtain metabolite profiles for urine, feces, and plasma.

5. Calculations and Statistics

The means and individual values were presented. Both individual and mean values were used to estimate pharmacokinetic parameters. Half-life was expressed as an apparent half-life and estimated by least squares analysis. Area-under-the-curve was determined by the trapezoidal rule and curve-fitting was by simple linear regression.

II. RESULTS

A. DISTRIBUTION/EXCRETION STUDIES

1. Mass balance

Overall recovery of administered radioactivity was 94.5% (average of two dogs tested). Both urinary and fecal excretion were major routes of elimination (Table 2.). Individual variability for the two dogs was minimal. Recovery of radioactivity from individual matrices (urine, feces, plasma) was provided in the study report and shown to be acceptable (i.e., 97-102%).

TABLE 2. Recovery of Administered Radioactivity (% of dose) in Dogs 168 Hours Following a Single Oral Dose of [∝-^{14}C]XRD-537 BE[a]

Matrix	Dog 1	Dog 2	Average
Urine	42.5	43.9	43.2
Feces	50.6	48.6	49.6
Cage wash	2.1	1.3	1.7
Tissues/carcass	_[b]	_[b]	_[b]
Total	95.2	93.8	94.5

[a] Average of two dogs.
[b] Not measured
Data taken from Tables 1-1 and 1-2, pp. 60 and 63, MRID 45000425.

2. Absorption

Absorption of the test material may be implied from the urinary excretion and cage wash data as well as plasma concentrations. Based upon the urinary excretion/cage wash data, approximately 50% of the administered oral dose was absorbed. In the absence of biliary excretion data, it is not possible to determine with certainty if the radioactivity eliminated in the feces represented previously absorbed or unabsorbed radioactivity.

Based upon time-course data for plasma radioactivity, absorption of the test material following oral administration was rapid and reached maximum concentrations within one to two hours.

3. Excretion

Both urinary and fecal elimination were major routes of excretion accounting for approximately 43% and 50%, respectively, of the administered dose over a 168-hour period (Table 2). Time course for these routes of elimination are shown in Tables 3 and 4. Greater than 90% of the urinary elimination occurred within 72 hours in both dogs. The time-course for fecal elimination was remarkably similar to that of urinary excretion with 85-90% completion within 72 hours (Table 4). There was very little individual variability in both urinary and fecal excretion.

TABLE 3. Cumulative Urinary Excretion (% of dose) of [α-^{14}C]XRD-537 BE in Dogs.

Time (hrs)	Dog 1	Dog 2
0-4	2.9	3.6
8	5.5	17.9
12	25.9	32.3
24	27.0	34.6
48	35.1	40.4
72	39.1	42.3
96	41.0	43.1
120	41.8	43.5
144	42.3	43.8
168	42.5	43.9

Data taken from Table 1-1, p. 60, MRID 45000425; does not include cage wash data.

TABLE 4. Cumulative Fecal Excretion (% of dose) of [∝-^{14}C]XRD-537 BE in Dogs.		
Time (hrs)	Dog 1	Dog 2
0-4	0.0	0.0
8	0.0	0.0
12	20.2	1.3
24	28.3	23.8
48	37.2	37.5
72	43.3	43.7
96	46.4	46.4
120	49.1	47.4
144	49.9	48.2
168	50.6	48.6

Data taken from Table 1-1, p. 60, MRID 45000425.

4. Tissue distribution

Tissue burdens and distribution of radioactivity were not assessed in the reviewed study. Based upon radioactivity recovered in excreta, tissue burdens would be minimal at 168 hours and greater.

B. PHARMACOKINETIC STUDIES

Evaluation of kinetic parameters was limited to blood and plasma time-course assessments (Table 5). Kinetic parameters are summarized in Table 6.

TABLE 5. Time-course of Radioactivity in Blood and Plasma of Dogs Following a Single Oral Dose of [∝-^{14}C]XRD-537 BE[a]		
Time (hrs)	**Blood**	**Plasma**
0.25	1.48	2.32
0.5	1.56	2.55
1	1.69	2.84
2	1.79	2.88
3	1.46	2.55
4	1.35	2.26
6	0.57	1.04
8	0.45	0.80
12	0.33	0.58
24	0.34	0.61
48	0.16	0.29
96	0.03	0.06
120	0.02	0.04
168	0.01	0.01

[a] Expressed as μg eq. XRD-537 BE/mL; average value for two dogs
Data taken from Table 2-2, p. 68, MRID 45000425.

TABLE 6. Pharmacokinetic Parameters for [∝-^{14}C]XRD-537 BE in Blood and Plasma of Dogs Following a Single Oral Dose (1 mg/kg).		
Parameter	**Dog 1**	**Dog 2**
$t_{1/2}$ blood	22 hrs	23 hrs
$t_{1/2}$ plasma	20 hrs	25 hrs
t_{max} blood	1 hr	2 hrs
t_{max} plasma	1 hr	2 hrs
C_{max} blood	1.78 μg eq./mL	1.87 μg eq./mL
C_{max} plasma	2.95 μg eq./mL	2.97 μg eq./mL
AUC blood	29.8 μg eq. · hr/mL	23.4 μg eq. · hr/mL
AUC plasma	52.7 μg eq. · hr/mL	41.0 μg eq. · hr/mL

Data taken from Table 2-1, pp. 65 67 MRID 45000425.

Time-course analysis of blood/plasma radioactivity revealed relatively rapid uptake with maximum concentrations attained at 1-2 hours for both blood (1.78 - 1.87 μg eq./mL) and plasma (2.95 - 2.97 μg eq./mL). Clearance from both the plasma and blood was not especially rapid but near complete at 48 hours. A graphic display (in the study report MRID 45000425) of data from the two dogs suggested a biphasic clearance with a slight plateau or spike at approximately 24-36 hours. There was measurable radioactivity in both matrices at 168 hours indicative of very low concentrations (≤0.01 μg eq./mL). Area-under-the-curve (AUC) was approximately 1.75-fold greater for plasma than for whole blood suggesting that no binding to red blood cells.

C. METABOLITE CHARACTERIZATION STUDIES

Metabolite profiles were obtained for plasma, urine, and feces using TLC and HPLC techniques. Both quantitative and qualitative analyses were performed. Metabolites were identified by the matrix in which they were detected (U: urine, P: plasma, F; feces) and numerically based on increasing Rf value. An "a" designation indicated metabolites derived directly from [∝-^{14}C]XRD-537 BE, and "T" indicated detection by TLC.

For all three matrices, R-(+)-2-[4-cyano-2-fluorophenoxy)phenoxy]propanoic acid was the major metabolite. One other minor and uncharacterized metabolite was also found in plasma, feces, and urine, and several were found in two of the three matrices.

1. Urine

No parent compound was detected in the 0-48 urine samples of either dog. Six fractions were detected by TLC in the nonhydrolyzed urine samples. The major metabolite was R-(+)-2-[4-cyano-2-fluorophenoxy)phenoxy]propanoic acid which accounted for 29 and 31% of the administered dose (82.7% and 76.6% of the urinary radioactivity, respectively) in nonhydrolyzed urine samples from two dogs (Table 7). Only two other fractions accounted for more than 1% of the administered dose (DUa3T at 2.2 and 4.1% and DUa4T at 1.3 and 1.7% for the two dogs). Individual variability in the metabolite profiles for the two dogs tested was negligible.

TABLE 7. Urinary Metabolites (% of dose) in Dogs at 48 Hours Following a Single Oral Dose (1 mg/kg) of [∝-^{14}C]XRD-537 BE[a]

Metabolite	Dog 1	Dog 2	Average
XRD-537 BE	ND	ND	ND
R-(+)-2-[4-cyano-2-fluorophenoxy)phenoxy]propanoic acid	29.0	31.0	30.0
4-(2-fluoro-4-cyanophenoxyl)phenol	0.4	0.5	0.4
DUa1T (not identified)	0.6	0.5	0.5
DUa2T (not identified)	0.2	0.3	0.2
DUa3T (not identified)	2.2	4.1	3.2
DU4aT (not identified)	1.3	1.7	1.5
Others (not identified)	≈0.9	≈0.8	≈0.8

[a] Nonhydrolyzed samples
ND: not detected
Data taken from Table 6, p. 92, MRID 45000425

Enzymatic hydrolysis of urine samples slightly increased the amount of R-(+)-2-[4-cyano-2-fluorophenoxy)phenoxy]propanoic acid and slightly decreased DUa4T.

Results of HPLC analysis of urine samples were consistent with the TLC results (data not reproduced in this Data Evaluation Record). The prominent metabolites were R-(+)-2-[4-cyano-2-fluorophenoxy)phenoxy]propanoic acid (77.9% of the administered dose) and DUa2 (3.7% of the administered dose). The 4-(2-fluoro-4-cyanophenoxyl) phenol metabolite represented 0.6% of the administered dose. Enzymatic hydrolysis increased the acid metabolite, 4-(2-fluoro-4-cyanophenoxyl)phenol, and DUa3.

2. Feces

Five fractions were identified in 0-48 hour fecal samples (Table 8). Additionally, parent compound was detected in the fecal sample of one dog. Similar to the metabolite profiles for urine, the major metabolite was R-(+)-2-[4-cyano-2-fluorophenoxy)phenoxy]propanoic acid which accounted for 25.2 and 27.8% of the administered dose (67.8% and 74.2% of the fecal radioactivity, respectively) in nonhydrolyzed fecal samples from two dogs (Table 8). Small amounts of parent compound (0.9% of the administered dose) were detected in one dog but not the other. The metabolite, 4-(2-fluoro-4-cyanophenoxyl)phenol, represented 5.0 and 7.2% of the administered dose. Other fractions represented only minor percentages of the administered dose. No qualitative differences and only minor quantitative variability was observed in the fecal metabolite profiles of the two dogs.

TABLE 8. Fecal Metabolites (% of dose) in Dogs at 48 Hours Following a Single Oral Dose (1 mg/kg) of [∝-^{14}C]XRD-537 BE

Metabolite	Dog 1	Dog 2	Average
XRD-537 BE	0.9	ND	–
R-(+)-2-[4-cyano-2-fluorophenoxy)phenoxy]propanoic acid	25.2	27.8	26.5
4-(2-fluoro-4-cyanophenoxyl)phenol	5.0	7.2	6.1
DFa1T (not identified)	0.6	0.2	0.4
DFa2T (not identified)	0.7	0.3	0.5
DFa3T (not identified)	2.2	0.4	1.3
Others (not identified)	≈1.8	≈0.5	≈1.7

ND: not detected
Data taken from Table 7, p. 95, MRID 45000425

HPLC analysis revealed eight fractions (DFa6 was detected in only one of two dogs) and the parent compound as components in 0-48 hour fecal samples (data not reproduced in the Data Evaluation Record). Similar to TLC analysis, R-(+)-2-[4-cyano-2-fluorophenoxy)phenoxy]propanoic acid was the most prevalent metabolite representing 66% of the administered dose. Parent compound was detected but accounted for only <1% of the dose. The 4-(2-fluoro-4-cyanophenoxyl)phenol metabolite represented ≈5% of the administered dose. The remaining fractions each represented <5% of the administered dose.

3. Plasma

TLC analysis of plasma metabolites at four and eight hours after administration are summarized in Table 9. No parent compound was detected in either dog at either sampling time. Similar to both urine and feces, R-(+)-2-[4-cyano-2-fluorophenoxy)phenoxy]propanoic acid was the most prominent fraction, representing ~94% of the administered radioactivity (2.68 μg eq. XRD-537 BE/mL) in the 1- hour samples and decreasing to ~86% of the dose (1.95 μg eq. XRD-537 BE/mL) at four hours. With the exception of DPa4 which accounted for approximately 4.2% and 11.0% of the administered radioactivity at one and four hours, respectively, all other fractions represented <1% of the dose. There was no significant variability observed in the plasma metabolite profiles of the two dogs tested. Recovery of radioactivity in plasma was >99%. HPLC analysis (data not shown in this Data Evaluation Record) yielded very similar profiles with the exception of an additional fraction detected that represented ~0.8% of the administered radioactivity.

TABLE 9. Plasma Metabolites (% of dose) in Dogs at 4 and 8 Hours Following a Single Oral Dose (1 mg/kg) of [∝-^{14}C]XRD-537 BE

Metabolite	1-Hr			4-Hrs		
	Dog 1	Dog 2	Average	Dog 1	Dog 2	Average
XRD-537 BE	ND	ND	-	ND	ND	-
R-(+)-2-[4-cyano-2-fluorophenoxy)phenoxy] propanoic acid	91.7	97.1	94.4	80.0	92.3	86.1
4-(2-fluoro-4-cyanophenoxyl)phenol	ND	ND	ND	ND	ND	ND
DPa1T (not identified)	ND	ND	ND	ND	ND	ND
DPa2T (not identified)	0.2	0.4	0.3	0.2	0.6	0.4
DPa3T (not identified)	0.4	0.2	0.3	0.8	0.5	0.6
DPa4T (not identified)	6.8	1.6	4.2	17.3	4.7	11.0
DPa5T (not identified)	ND	ND	ND	ND	0.5	ND
Others (not identified)	0.6	0.5	0.5	1.4	1.2	1.5

ND: not detected ND or not determined
Data taken from Table 8, p. 98, MRID 45000425

III. DISCUSSION

A. DISCUSSION

In a metabolism and disposition study (MRID 45000425) two male beagle dogs were given [∝-^{14}C]XRD-537 BE (>97% radiochemical purity, Lot no. A-903-48, 2.01 MBq/mg) and nonlabeled XRD-537 (97% chemical purity, Lot no. AGR 295713) orally at a dose of 1 mg/kg. Blood, urine, and feces were collected up to 168 hours after dosing. Metabolite profiles and metabolite characterizations were performed for all three matrices.

No treatment-related adverse effects were reported for either of the two dogs. Overall recovery of administered radioactivity was an acceptable 93.8 and 95.2% for each of two dogs. Based upon urinary excretion and blood time-course data, approximately 50% of a single oral dose of the test article was absorbed with most absorption occurring over several hours. In the absence of biliary excretion data, it was not possible to reliably assess total absorption. Excretion via urine and feces were both major routes of elimination and quantitatively similar. Urinary excretion accounted for 42.5 and 43.9% of the administered dose over 168 hours and was 90% complete within 72 hours. Over 168 hours, fecal excretion accounted for 50.6 and 48.6% of the administered radioactivity in each of two dogs and was also 85-90% complete within 72 hours. Assessment of tissue burdens was not in the study protocol and, therefore, no data were available.

Time-course analysis of blood/plasma radioactivity revealed relatively rapid uptake with maximum concentrations attained at 1-2 hours for both blood (1.78 - 1.87 μg eq./mL) and plasma (2.95 - 2.97 μg eq./mL). Clearance from both the plasma and blood was not especially rapid but near complete at 48 hours. The limited data from only two dogs suggested a biphasic clearance with a slight plateau or spike at approximately 24-36 hours, possibly indicative of enterohepatic cycling or delayed release of compound-related radioactivity from a tissue site. There was measurable radioactivity in both matrices at 168 hours. Area-under-the-curve (AUC) was approximately 1.75-fold greater for plasma than for whole blood, possibly indicative of plasma protein binding.

This study provided data showing that at least 50% of a single oral dose (1 mg/kg) of XRD-537 BE to dogs is absorbed over a 168-hour period and that both the feces and urine are major routes of excretion. The test article appears to nearly complete metabolism primarily by hydrolysis to the major metabolite, R-(+)-2-[4-cyano-2-fluorophenoxy)phenoxy]propanoic acid which was found in both the urine and feces. Several other metabolites were also formed, each representing <5% of the administered dose. Only minimal amounts of parent compound were detected in the feces.

This metabolism study in rats is **Acceptable/Non-guideline**. The study used only two male dogs rather than four and did not provide data regarding tissue distribution. Therefore, it does not meet the minimum requirements for a Tier 1 metabolism study. Although not satisfying the requirements for a Metabolism and Pharmacokinetics Study [OPPTS 870.7485 (85-1)], this study (MRID 45000425) was properly conducted and reported, and provided useful data regarding the absorption, metabolism and excretion of XRD-537 BE in dogs following a single oral dose.

B. STUDY DEFICIENCIES

The 168-hr tabled value for radioactivity in the blood (Table 2-1, p. 65 of study report) in the study report is inconsistent with the graphic display (Figure 2-2, p. 67) which depicts the 120-hr value being the terminal reading. This minor inconsistency, however, did not compromise the validity of the study or the overall interpretation of data.

DATA EVALUATION RECORD

CYHALOFOP BUTYL

STUDY TYPE: METABOLISM AND PHARMACOKINETICS – RAT
OPPTS: 870-7485 (§85-1)
MRID 45000426, 45000427, 4500028
5

Prepared for

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Office of Pesticide Programs
U.S. Environmental Protection Agency
1921 Jefferson Davis Highway
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Prepared by

Chemical Hazard Evaluation Group
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Disclaimer

This review may have been altered subsequent to the contractor's signatures above.

Oak Ridge National Laboratory, managed by UTBattelle, LLC, for the U.S. Dept. of Energy under contract

CYHALOFOP BUTYL **Metabolism Study [OPPTS 870.7485 (§85-1)]**

EPA Reviewer: John Whalan, Date 5-7-01
Registration Action Branch 2 (7509C)
EPA Work Assignment Manager: Sanvyette Williams-Foy, D.V.M., Date 5/31/01
Registration Action Branch 2 (7509C)
TXR No: 0050348

DATA EVALUATION RECORD

STUDY TYPE: Metabolism - Rat [OPPTS 870.7485 (§85-1)]

DP BARCODE: D268553
P.C. CODE: 082583

SUBMISSION CODE: S
TOX. CHEM. NO.: NA

TEST MATERIAL (PURITY): XRD-537 BE (>97%)

SYNONYMS: R-(+)-n-butyl 2-[4-(2-fluoro-4-cyanophenoxy)phenoxy]propanoate);
Cyhalofop butyl

CITATION:

1. Ninomiya, S-i., et al. Stability, homogeneity of dose formulation of ^{14}C XRD-537 BE for animal metabolism studies (1995). Tokai Research Laboratories, Daiichi Pure Chemicals Co., Ltd., 2117, Muramatsu, Tokai-mura, Naka-gun Ibaraki 319-11, Japan. Laboratory Study ID GHF-R-296. MRID 45000528. March 3, 1995.Unpublished.

2. Ninomiya, S-i, et al. (1995). XRD-537 BE absorption, metabolism and excretion preliminary study in the Fischer rat. Tokai Research Laboratories, Daiichi Pure Chemicals Co., Ltd., 2117, Muramatsu, Tokai-mura, Naka-gun Ibaraki 319-11, Japan. Laboratory Study ID GHF-R-298. MRID 45000426. March 3, 1995.Unpublished.

3. Ninomiya, S-i, et al. (1995). XRD-537 BE absorption, metabolism and excretion study in the Fischer rat. Tokai Research Laboratories, Daiichi Pure Chemicals Co., Ltd., 2117, Muramatsu, Tokai-mura, Naka-gun Ibaraki 319-11, Japan. Laboratory Study ID GHF-R-297. MRID 45000427. March 3, 1995.Unpublished.

SPONSOR: Dow Chemical Japan Limited, Nichimen Corporation

EXECUTIVE SUMMARY: A preliminary study (MRID 45000426) and a main study (MRID 45000427) were conducted to evaluate the metabolism and disposition of XRD-537 BE in male and female Fischer rats. Non-labeled XRD-537 BE (Lot no. AGR 295713, purity >97%) and radiolabeled XRD-537 BE ([α-^{14}C] XRD-537 BE, Lot no. A-903-48 or [β-^{14}C] XRD-537 BE, Lot no. A-903-34a, >97% radiochemical purity for both label positions) was administered orally to groups of five to seven male and female Fischer rats. Dose groups in the main study (MRID 45000427) included single low dose (1 mg/kg), single high dose (50 mg/kg), and a 14-day repeated low dose (1 mg/kg/day) and were used to assess mass balance, pharmacokinetics, biliary excretion, and metabolite profiles. The preliminary study using the same test articles assessed

mass balance, pharmacokinetics, biliary excretion, and metabolite profiles in groups of two to four male and female Fisher rats given a single 1 mg/kg dose. Stability and homogeneity of the dose formulation was assessed in a separate study (MRID 45000528).

Mass balance in both the preliminary and main study was acceptable with overall recovery of administered radioactivity ranging from 94-104%. Based upon excretion and tissue burden data, absorption of the test material was shown to be 93-100% and was consistent in both the preliminary and main study, among the various dose groups and for both label positions.

Urinary excretion was the major route of elimination regardless of dose, label position, or gender. Over a 168-hour period, 84-100% of the administered radioactivity was eliminated via the urine with 86-90% eliminated within 24 hours. The feces represented a minor route of excretion (<5% of the administered dose) over the 168-hour time period. There was no evidence for expired air as a route of elimination.

There was considerable individual variability in biliary excretion disallowing valid assessment of gender-related or label position-related differences in biliary elimination. Results of the preliminary and main studies showed that over a 24-hour period, biliary elimination accounted for 1.7 % and 20.1% of the administered dose in males and females, respectively, in the low-dose [α-^{14}C]XRD-537 BE group, and 17.0% (males) and 11.6% (females) of the administered dose in the [β-^{14}C]XRD-537 BE low-dose group.

Tissue distribution studies indicated that the greatest radioactivity over 24 hours occurred in the liver, kidneys, plasma, whole blood, heart, lung, and stomach. The highest tissue concentrations (2-hr liver and kidney) represented 15-19% of the administered dose while most tissue levels accounted for <1%. Tissue/organ burdens appeared somewhat less in females while concentrations in gastrointestinal organs were somewhat greater, suggesting decreased absorption. Consistent with the rapid excretion of test article-related radioactivity, tissue/organ levels of radioactivity notably declined to near detection limits by 24 hours in all dose groups.

Blood concentration time-course data from both the preliminary and main studies showed a biphasic pattern for both [α-^{14}C]XRD-537 BE and [β-^{14}C]XRD-537 BE. There were no substantial differences in pharmacokinetic indices (C_{max}, t_{cmax}, $t_{1/2}$, AUC) between the two label positions based upon data from the preliminary study. Time-to-maximum plasma concentration (t_{cmax} of 0.5 to 4 hrs) and elimination half-times ($t_{1/2}$ of 1.4-7.9 hrs) reflected the relatively rapid absorption. There was evidence for gender-related differences, however, with females exhibiting somewhat shorter t_{cmax} and lower C_{max} values suggestive of saturated absorption processes. This was also reflected in a two- to four-fold lower AUC values for females.

Metabolite characterization revealed that the acid metabolite (R-(+)-2-[4-(4-cyano-2-fluoro-phenoxy)phenoxy]propanoic acid) was the most prominent fraction in plasma representing ~90-94% of the plasma activity for males and ~75-81% for females regardless of dose. No parent compound or other metabolites were detected. There were no appreciable differences in the quantitative or qualitative plasma metabolite profiles between males and females. Urinary and fecal metabolite profiles also showed that the acid metabolite was the most prevalent biotransformation product, accounting for approximately 71-87% (urine) and 46-75% (feces) of the activity in those matrices. Somewhat greater levels of uncharacterized components were

detected in the urine and feces of females resulting gender-related variability in metabolite profiles.

These metabolism/kinetics studies in rats are collectively **Acceptable/Guideline** and satisfy the requirements for a Metabolism and Pharmacokinetics Study [OPPTS 870.7485 (§85-1)].

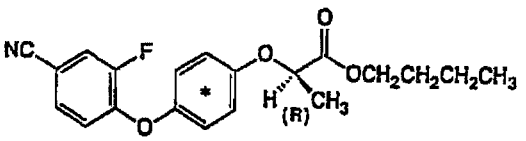
COMPLIANCE: Good Laboratory Practice Compliance Statements, and signed and dated Quality Assurance statements were provided in the study reports.

I. MATERIALS AND METHODS

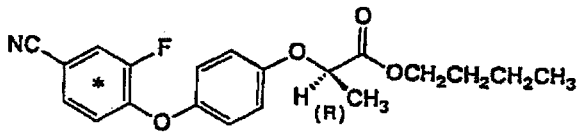
A. MATERIALS

1. Test compound

Radiolabeled:[α-^{14}C] XRD-537 BE; [β-^{14}C] XRD-537 BE
Lot No.: A-903-48; A-903-34a for all studies
Specific Activity: 2.008 MBq/mg; 2.692 MBq/mg for all studies
Radiochemical purity: >97% for both label positions
Description: not provided
Contaminants: none specified
Structure:



[α-^{14}C] XRD-537 BE



[β-^{14}C] XRD-537 BE

Non-radiolabeled: XRD-537 BE
Lot No.: AGR 295713 for all studies
Purity: >97%
Description: not provided
Contaminants: none specified
CAS No.: 122008-85-9

2. Vehicle

Distilled water (lot no. 9008ST; Hikari Pharmaceutical Co., Ltd. and lot no. 2G75N; Otsuka Pharmaceutical Co., Ltd.); carboxymethylcellulose sodium (lot no.

WDP1183;Wako Pure Chemical Industries); Tween 80 (lot no. TWP5590; WDP1183;Wako Pure Chemical Industries).

3. Test animals

Species: Rat for all studies
Strain: Fischer 344 for all studies (Charles River Japan)
Age and weight at study initiation:
1) 8 weeks: 177.9 - 199.0 g (males); 130.1 - 134.4 g (females)
2) 8 weeks: 189.5 - 214.1 g (males; 123.1 - 144.0 g (females)
Housing: During most phases of the experiment, rats were housed individually in glass metabolism cages. For blood concentration experiments, rats were housed two/sex in stainless steel cages.
Diet: Diet for rodents (MF, Oriental Yeast Co., Ltd.) was provided *ad libitum.*
Water: Water analyzed for impurities (Tokyo Technical Services) was provided *ad libitum.*
Environmental conditions:
Temperature: 23±2°C
Humidity: 55±15%
Air changes: at least 15/hr
Photoperiod: 12 hrs/12 hrs
Acclimation period: 8 - 12 days for all studies

4. Preparation of dosing solution

For stability and homogeneity assessment (MRID 45000528), solutions were prepared by mixing appropriate amounts of labeled and non-labeled test article with 0.5% carboxymethylcellulose and Tween 80 (2% final concentration) followed by sonication for 30 minutes and stirring for 15 minutes. Homogeneity was evaluated for both suspension concentrations (0.5 and 25 mg/mL) of both label positions ([α-^{14}C] and [β-^{14}C]) at 0, 5, 10, 15, and 30 minutes.

Results –
Homogeneity: Homogeneity of [α-^{14}C]XRD-537 BE dose suspensions was >98.9% for the 0.5 mg/mL suspension at all time points and 60.7-99.8% for the 25 mg/mL suspension. For [β-^{14}C]XRD-537 BE, homogeneity was 85.5-103.0% for the 0.5 mg/mL concentration and 78.1-100.9% for the 25 mg/mL concentration. Results of the stability/homogeneity study showed that the test article suspensions were best quantified for dosing by volume rather than by weighing, the latter allowing greater time for partitioning of the vehicle and the test article.

Stability: HPLC and radioactivity analysis confirmed chemical and radiochemical stability of both the 0.5 mg/mL and 25 mg/mL concentrations of XRD-537 suspensions in 0.5% methylcellulose for up to 21 days (MRID 45000528).

Dose confirmation: Dose confirmation was provided in MRID 45000528 consistent with a protocol amendment regarding addition of 2% Tween 80 to the suspension and radioactivity determinations in triplicate samples.

B. STUDY DESIGN AND METHODS

1. Group arrangements

The experimental groups established for the metabolism studies (MRID 45000426, 45000427) are summarized in Table 1. Rats nearest the mean body weight were randomly assigned to the groups following acclimation. Rats outside of the defined body weight range (180 ±20% for males and 120±20% for females) were excluded from the study.

TABLE 1. Study design for disposition and metabolism of XRD-537 BE in rats

Experiment group	Dose (mg/kg)	Number/Sex	Remarks
Preliminary			Preliminary study; single oral low dose of [α-^{14}C]XRD-537 BE or [β-^{14}C]XRD-537 BE (MRID 45000426)
B1	1	2M; 2F	[α-^{14}C] XRD-537 BE; mass balance
B2	1	2M; 2F	[β-^{14}C] XRD-537 BE; mass balance
B3	1	2M; 2F	[α-^{14}C] XRD-537 BE; pharmacokinetics
B4	1	2M; 2F	[β-^{14}C] XRD-537 BE; pharmacokinetics
B5	1	2M; 2F	[α-^{14}C] XRD-537 BE; biliary secretion
B6	1	2M; 2F	[β-^{14}C] XRD-537 BE; biliary secretion
B7	1	2M; 2F	[α-^{14}C] XRD-537 BE; metabolite profile
B8	1	2M; 2F	[β-^{14}C] XRD-537 BE; metabolite profile
		6M; 6F	additional rats used for analysis of parent compound
Main study Low dose			Main study (MRID 45000427)
B1	1	5M; 5F	[α-^{14}C] XRD-537 BE; mass balance
B5	1	5M; 5F	[α-^{14}C] XRD-537 BE; pharmacokinetics
B9	1	5M; 5F	[α-^{14}C] XRD-537 BE; tissue/organ distribution at 2 hrs
B10	1	5M; 5F	[α-^{14}C] XRD-537 BE; tissue/organ distribution at 6 hrs
B11	1	5M; 5F	[α-^{14}C] XRD-537 BE; tissue/organ distribution at 24 hrs
B12	1	5M; 5F	[α-^{14}C] XRD-537 BE; tissue/organ distribution at 168 hrs
B35	1	5M; 5F	[α-^{14}C] XRD-537 BE; biliary excretion
B36	1	5M; 5F	[β-^{14}C] XRD-537 BE; biliary excretion
Main study High dose			Main study (MRID 45000427)
D2	50	5M; 5F	[α-^{14}C] XRD-537 BE; mass balance
D6	50	5M; 5F	[α-^{14}C] XRD-537 BE; pharmacokinetics
D13	50	5M; 5F	[α-^{14}C] XRD-537 BE; tissue/organ distribution at 4 hrs
D14	50	5M; 5F	[α-^{14}C] XRD-537 BE; tissue/organ distribution at 9 hrs
D15	50	5M; 5F	[α-^{14}C] XRD-537 BE; tissue/organ distribution at 24 hrs
D16	50	5M; 5F	[α-^{14}C] XRD-537 BE; tissue/organ distribution at 168 hrs
Main study Repeat dose			Main study (MRID 45000427)
C25	1	7M; 7F	14-day repeated oral low dose of non-labeled XRD-537 BE followed by single [α-^{14}C] XRD-537 BE dose on Day 15 (5 rats of each sex)
C27	1	7M; 7F	14-day repeated oral low dose; pharmacokinetics
C29	1	7M; 7F	14-day repeated oral low dose; tissue/organ distribution; 2 hrs
C30	1	7M; 7F	14-day repeated oral low dose; tissue/organ distribution; 6 hrs
C31	1	7M; 7F	14-day repeated oral low dose; tissue/organ distribution; 24 hrs

Information taken from p. 62, MRID 45000426 and pp. 47-48 of MRID 45000427.

2. Dosing and sample collection

In the preliminary study (MRID 45000426), non-fasted rats were given a single oral dose (1 mg/kg; ~2.0 MBq/kg; 2 mL/kg) of the test article (both labels) using a gas-tight Hamilton syringe. Rats in the main study were dosed similarly but two dose levels (1 and 50 mg/kg) were used as well a multiple (14-day) dose protocol. Rats were housed individually in glass metabolism cages during the experimental period. The cages were washed with 20 mL of distilled water following collection of feces. Samples were stored at -80°C until analyzed.

Expired air – Expired air was collected at 3, 6, 12 and 24 hours and at 24-hour intervals thereafter to 168 hours. Volatiles were trapped in two 200 mL traps containing methyl cellosolve and CO_2 was trapped in two 200 mL traps containing 20% monomethanolamine. The traps were replaced after each sampling.

Blood – Blood samples were taken via the caudal vein with heparinzed micropipettes at 15 and 30 minutes, and at 1, 2, 3, 4, 6, 8, 12, 24, 48, 96, 120, and 168 hours after dosing.

Bile – Bile was collected via a PE-10 cannula (fitted while rats were anesthetized but collection performed on unanesthetized rats) at 1, 2 ,3 4, 5, 6, 12, and 24 hours

Urine – Urine samples were collected in ice-cooled containers at 3, 6, 12 and 24 hours and at 24-hour intervals thereafter to 168 hours. Urine was collected from rats fitted with bile duct cannulae at 6, 12, and 24 hours.

Feces – Feces were collected in ice-cooled containers at 3, 6, 12 and 24 hours and at 24-hour intervals thereafter to 168 hours. Feces were collected from rats fitted with bile duct cannulae at 6, 12, and 24 hours.

Cage wash – Cages were washed with ~50 mL of distilled water after each sample collection and retained for analysis.

Tissues/Carcass – Carcasses were weighed, processed, and retained for analysis. For tissue collection

3. Sample preparation/analysis

Expired air – One mL aliquots of methyl cellosolve from volatile organics traps were mixed with 10 mL of Atomlight and analyzed for radioactivity. One mL aliqouts of monomethanolamine from the CO_2 traps were mixed with 10 mL of Hionicfluor (Packard) and analyzed for radioactivity.

Blood – Samples were dissolved in 2 mL of Soluene and were decolorized with 0.4 mL of saturated benzoyl peroxide in benzene. The samples were then mixed with 13 mL of Econofluor and analyzed for radioactivity.

Bile – Bile samples were diluted with 25 mL of distilled water and 1 mL aliquots were mixed with 10 mL of Atomlight and analyzed for radioactivity.

Urine – Urine samples were diluted with distilled water to 100 mL and 1 mL aliquots mixed with 10 mL of scintillation fluid (Atomlight, DuPont NEN Research Products). Samples of this mixture were analyzed for radioactivity.

Feces – Fecal samples were mixed with distilled water to 300 mL, stirred and homogenized. Twenty mL of Soluene-350 (Packard) was added to 1 mL aliquots of

the homogenate and 13 mL of scintillation fluid (Econofluor, DuPont NEN Research Products) added.

Cage wash – Distilled water to 100 mL was added to cage wash samples and the mixtures homogenized. Ten mL of Atomlight scintillation fluid was added to 1 mL aliquots of the cage wash samples and the samples analyzed for radioactivity.

Tissues/Carcass – Carcasses were dissolved in 500 mL of 0.5 M sodium hydroxide and 80 mL of toluene for 72 hours. Following addition of distilled water (to 800 mL), the mixture was homogenized and 10 mL Atomlight was added to 0.5 mL aliquots of the homogenate and these samples analyzed for radioactivity. Tissues (liver and kidney) were homogenized in distilled water and volumes adjusted to 20 mL (kidney) and 50 mL (liver).

4. Analytical techniques

Liquid Scintillation Counting (LSC) – For stability and homogeneity evaluation (MRID 45000528), Aloka LSC-903 or Packard TR2500 instruments were used. ATOMLIGHT scintillation fluid (10 mL) was added to samples which were counted in duplicate or triplicate for 2-minute counts. Background was determined in duplicate for each sample batch using untreated control rats. The detection limit was set equivalent to twice background.

Preparation of analytical samples – two extraction methods were examined as briefly described below. Because of greater recovery efficiency, the solid-phase method was preferred.

Solid-phase extraction – Plasma, tissue and fecal homogenates, urine, and bile samples were separated using a Bond Elute C_{18} cartridge. To improve recovery of radioactivity in plasma samples, a WP™ C4 cartridge (Baker Co., Ltd.) was connected in series. Samples were mixed with acetate buffer prior to injection into the HPLC. Extraction efficiency was shown to be >95% for plasma, >90% for urine and bile, >85% for feces, >89% for liver samples, and >84% for kidney samples.

Liquid-phase extraction – Urine, bile, fecal, and tissue (liver and kidney) samples were also extracted with methanol, sonicated for 10 minutes, and centrifuged (procedure repeated twice). The pooled supernatants from each sample were adjusted to a fixed volume with additional methanol and analyzed for radioactivity. Recovery of radioactivity was >96% for urine and bile, >79% for fecal samples, >98% for liver, and >99% for kidney.

High Performance Liquid Chromatography (HPLC) – For stability analysis, a Novapak C_{18} column with a mobile phase of 0.5% acetic acid/water and 0.5% acetonitrile/water, flow rate of 1 mL/minute, and detection at 254 nm.

Thin-layer Chromatography (TLC) – TLC utilized silica gel 60 F_{254} plates (E. Merck Co.) developed two dimensionally with various solvent systems. The solvent systems providing the best separation were: toluene/ethyl acetate/methanol/triethylamine (15:10:5:1, v/v/v/v) and toluene/ethyl acetate/methanol/acetic acid (15:10:5:1, v/v/v/v). Developed TLC plates were placed in contact with imaging plates (Type-BA, Fuji Film), exposed for 24 hours, and analyzed with a Fujix BA100 (Fuji Film) bio-imager.

Volatile metabolite assessment – Urine and bile samples were analyzed for volatile metabolites by comparison of radioactivity before and after drying of the samples in a rotary evaporator. Lyophilized samples were dissolved in 1 mL of distilled water and 10 mL of Atomlight for radioactivity determination.

6. Calculations and Statistics

Group means and standard errors were calculated and presented along with individual animal data.

II. RESULTS

A. DISTRIBUTION/EXCRETION STUDIES

1. Mass balance

Overall recovery of administered radioactivity was approximately 94 to 104% for the preliminary study (MRID 45000426) (Table 2). Neither gender nor label position appeared to affect overall recovery of radioactivity. For the main study, overall recovery of administered radioactivity was >94%. Mass balance data for the main study are summarized in Table 3.

TABLE 2. Overall recovery of administered radioactivity (% of dose) at 168 hours post-dosing in rats given a single oral dose of [α-^{14}C]XRD-537 BE or [β-^{14}C]XRD-537 BE (Preliminary study[a])

Matrix	[α-^{14}C]XRD-537 BE		[β-^{14}C]XRD-537 BE	
	Males	Females	Males	Females
Expired air	ND	ND	ND	ND
Urine	100.2/97.8	93.2/99.5	93.2/93.5	95.6/97.2
Feces	3.2/2.8	1.0/1.1	4.1/4.2	1.5/1.6
Tissues/Carcass	0.5/0.5	0.0/0.0	0.5/0.6	0.0/0.0
Cage wash	0.5/0.9	0.2/0.5	0.6/1.1	0.2/0.2
Total	104.4/102.0	94.4/101.1	98.4/99.4	97.3/99.0

[a] Data for two rats of each sex.
ND: no radioactivity detected in volatile organics or as $^{14}CO_2$.
Data taken from Tables 1-1, 2-1, 3-1, 4-1, pp. 147, 152, 157, and 162, MRID 45000426.

TABLE 3. Overall recovery of administered radioactivity (% of dose) at 168 hours following oral administration of [^{14}C]XRD-537 BE to rats (Main Study)

	Single low dose		Single high dose		Repeated low dose	
	Male	Female	Males	Females	Males	Females[a]
Expired air	ND	ND	ND	ND	ND	ND
Urine	93.8	94.3	90.2	91.7	91.0	86.4
Feces	4.1	1.3	4.4	1.6	2.5	2.0
Tissues/Carcass	0.2	0.1	0.6	0.0	1.7	0.1
Cage wash	0.7	1.6	0.5	0.7	0.9	5.8
Total	98.7	97.3	95.6	94.0	95.3	94.3

[a] Summary includes outlier data from rat #254.
ND: no radioactivity detected in volatile organics or as $^{14}CO_2$.
Data taken from Tables 1 (p. 113), 2 (p. 115), 3 (p.117), 17 (p. 161), 18 (p. 163), 17A-17C (pp. 304-306), and 18A-18C (pp. 307-309) of MRID 45000427.

2. Absorption

Absorption of the test material may be implied from the urinary excretion and cage wash data. The majority of the administered radioactivity was recovered in the urine in both the preliminary (93-100%) and main studies (94-99%). Therefore, it is evident that absorption was nearly complete over 168 hours following a single oral dose of 1 mg/kg or 50 mg/kg.

3. Excretion

Urinary excretion was the major route of elimination of radioactivity associated with either [α-^{14}C]XRD-537 BE or [β-^{14}C]XRD-537 BE and accounted for ~93 to 100 % of the administered 1 mg/kg dose in the preliminary study (Data not reproduced in this Data Evaluation Record). Time-course analysis of urinary excretion data from the preliminary study showed that >90% of the urinary excretion in both males and females occurred within 24 hours. Label position did not appear to affect urinary excretion profiles. Fecal elimination was minimal (~1 to 4%) in the preliminary study and there was no evidence for excretion via expired air.

Results of the main study affirmed urinary excretion as the major route of elimination of orally administered XRD-537 BE. Following administration of a single low dose (1 mg/kg), single high dose (50 mg/kg), or 14-day repeated low doses. Greater than 94% of the dose was eliminated in the urine over 168 hours with most (~90%) excretion occurring within 24 hours for all treatment groups (Table 4).

TABLE 4. Cumulative urinary excretion (% of dose) in rats following oral administration of [α -14C]XRD-537 BE						
Time (hrs)	Single low dose		Single high dose		Repeated dose males	Repeated dose females
	Males	Females	Males	Females		
0-3	23.5	26.4	9.0	19.7	27.7	38.0
6	45.9	60.0	35.8	65.4	47.6	72.5
12	82.0	89.3	78.2	88.2	83.0	80.4
24	93.1	93.6	89.2	91.3	90.0	85.5
48	93.6	94.0	89.9	91.6	90.6	86.1
72	93.7	94.0	90.0	91.7	90.7	86.2
96	93.7	94.0	90.1	91.7	90.8	86.3
120	93.8	94.0	90.1	91.7	90.9	86.3
144	93.8	94.2	90.1	91.7	90.9	86.4
168	93.8	94.3	90.2	91.7	91.0	86.4
Total	93.8	94.3	90.2	91.7	91.0	86.4

Data taken from Tables 1 (p. 113), 2 (p. 115), 3 (p. 117), 4 (p. 119), 17 (p. 161) and 18 (p. 163), MRID 45000427.

Similar to the findings in the preliminary study, results from the main study showed that fecal excretion contributed minimally to the overall elimination of test article-related radioactivity for all treatment groups (data not shown). Fecal excretion was essentially complete at 48 hours after dosing regardless of treatment group.

Over a 24-hour period, biliary elimination of radioactivity in the preliminary experiment ranged from 1.7 to 20.1% of the administered dose (Table 5). There was considerable individual variability in biliary excretion. This, and the limited number of experimental animals, made it difficult to assess possible gender-related or label position-related differences in biliary elimination.

TABLE 5. Cumulative biliary elimination of radioactivity (% of dose) over 24 hours in rats given a single oral dose (1 mg/kg) of [α-^{14}C]XRD-537 BE or [β-^{14}C]XRD-537 BE (Preliminary Study)[a]				
Time (hrs)	[α-^{14}C]XRD-537 BE		[β-^{14}C]XRD-537 BE	
	Males	Females	MalesFemales	Females
0-1	0.5/1.2	1.8/0.2	0.5/1.2	0.8/2.1
2	1.6/4.6	5.7/0.5	1.8/3.2	2.5/7.5
3	2.3/5.2	9.0/0.7	3.3/4.9	3.8/11.4
4	2.8/5.5	12.7/0.8	4.6/6.2	4.7/13.5
5	3.1/5.5	15.4/0.9	5.6/7.3	5.6/14.5
6	3.3/5.6	17.1/1.0	6.3/8.0	6.3/14.9
12	4.1/7.6	19.9/1.1	9.8/11.4	9.5/16.0
24	5.3/8.6	20.1/1.7	16.5/17.3	11.9/16.5

[a] Data for two rats of each sex.

Data taken from Tables 9-1, 10-1, 11-1, 12-1, pp. 183, 190, 197, and 204, MRID 45000426.

Biliary excretion over 24 hours in the main study is summarized in Table 6. Over a 24-hour period, biliary elimination accounted for 23.7 % and 18.4% of the administered dose in males and females, respectively, in the low-dose [α-^{14}C]XRD-537 BE group. For the groups receiving a single low dose of [β-^{14}C]XRD-537 BE, 24-hour

biliary elimination accounted for 17.0% (males) and 11.6% (females) of the administered dose.

TABLE 6. Cumulative biliary elimination of radioactivity (% of dose) over 24 hours in rats given a single oral dose of [α-^{14}C]XRD-537 BE or [β-^{14}C]XRD-537 BE (Main study)

Time (hrs)	[α-^{14}C]XRD-537 BE		[β-^{14}C]XRD-537 BE	
	Males	Females	Males	Females
0-1	1.2 ± 0.3	1.0 ± 0.4	0.6 ± 0.5	0.5 ± 0.5
2	3.7 ± 0.9	4.1 ± 1.8	1.8 ± 1.6	1.9 ± 1.9
3	6.9 ± 1.6	6.5 ± 3.0	3.2 ± 2.5	4.2 ± 4.0
4	9.4 ± 2.4	8.8 ± 4.4	4.8 ± 3.6	5.2 ± 4.9
5	11.4 ± 3.4	10.5 ± 5.5	5.3 ± 4.0	6.4 ± 5.9
6	13.2 ± 4.4	11.4 ± 6.0	6.2 ± 4.4	7.3 ± 6.5
12	18.2 ± 6.3	5.2 ± 7.4	9.4 ± 4.4	10.0 ± 7.5
24	23.7 ± 6.0	18.4 ± 7.1	17.0 ± 6.7	11.6 ± 8.4

Data taken from Tables 9-1, 10-1, 11-1, 12-1, pp. 129, 132, 135, and 138, MRID 45000427.

4. Tissue distribution

Results of the preliminary study revealed radioactivity in the liver and kidneys (the only tissues for which distribution data were acquired) to be minimal (<0.5% of the administered dose).

Results of the main study showed that the plasma, liver and kidneys had the highest levels of radioactivity at 0.5 - 24 hours after administration. Heart, lung, and stomach also exhibited high levels of radioactivity (Tables 7 and 8). The highest tissue concentrations (i.e., 2-hr liver and kidney) represented 15-19% of the administered dose while most tissues accounted for <1% of the administered dose. Tissue/organ burdens appeared to be somewhat less in females while concentrations in gastrointestinal organs were somewhat greater. Not all the concentrations for tissues/organs reflected the 50-fold difference in dose. Organs associated with metabolism/excretion processes (i.e., liver, kidneys, gastrointestinal tract) exhibited disproportionately low μg eq./g values. Consistent with the rapid excretion of test article-related radioactivity, tissue/organ levels of radioactivity notably declined to near detection limits at 24 hours. There was no evidence of sequestration in either the low- or high-dose group. Tissue/organ burdens for the repeat dose group (data not reproduced in this Data Evaluation Record) were similar to that observed for the single oral low dose indicating that the 14-day repeated dose treatment did not result in sequestration of test article or its metabolites. Clearance of radioactivity from the tissues was similar to that for the single low-dose group in that levels were near or below detection limits at 24 hours.

TABLE 7. Radioactivity (μg eq./g) in tissues/organs of rats given a single 1 mg/kg oral dose of [α-^{14}C]XRD-537 BE						
Tissue/Organ	2-hrs	0.5-hr	6-hrs	3-hrs	24-hrs	
	Males	Females	Males	Females	Males	Females
Plasma	2.61	1.39	1.79	0.45	0.04	0.01
Whole blood	1.48	0.83	1.01	0.26	0.02	0.0
Thyroid	0.38	0.17	0.41	0.06	ND	ND
Heart	0.42	0.22	0.31	0.07	0.01	ND
ND Lung	0.56	0.29	0.40	0.09	0.01	ND
Liver	3.88	2.02	2.94	0.87	0.07	0.02
Kidney	5.13	4.05	3.62	0.30	0.10	0.03
Stomach	1.96	3.53	0.79	1.51	0.01	0.01

ND: not detected
Data taken from Tables 13-1 and 14-1, pp. 141-142, and 146-147, MRID 45000427.

TABLE 8. Radioactivity (μg eq./g) in tissues/organs of rats given a single 50 mg/kg oral dose of [α-^{14}C]XRD-537 BE						
Tissue/Organ	9-hrs	2-hrs	9-hrs		24-hrs	
	Males	Females	Males	Females	Males	Females
Plasma	126.20	42.48	44.89	13.45	1.03	0.31
Whole blood	73.06	24.94	27.21	7.98	0.54	ND
Thyroid	17.93	5.39	4.70	ND	ND	ND
Heart	21.33	6.91	7.45	1.90	0.15	ND
Lung	28.18	8.83	10.17	2.94	0.23	ND
Liver	103.85	50.79	53.03	19.77	2.27	0.71
Kidney	99.32	83.92	56.33	30.52	3.14	1.15
Stomach	35.65	52.52	20.48	6.13	0.39	0.39

ND: not detected
Data taken from Tables 15-1 and 16-1, pp. 150-151 and 156-157, MRID 45000427.

B. PHARMACOKINETIC STUDIES

Blood concentration time-course data from the preliminary study showed a biphasic pattern for both [α-^{14}C]XRD-537 BE and [β-^{14}C]XRD-537 BE. There were no substantial differences in pharmacokinetic indices (C_{max}, t_{cmax}, $t_{1/2}$, AUC) between the two label positions based upon data from the preliminary study (Table 9). Gender-related differences were observed with females exhibiting somewhat shorter t_{cmax} and lower C_{max} values suggestive of saturated absorption processes. This was also reflected in two to four-fold lower AUC values for females.

Blood pharmacokinetic data for the experimental groups in the main study are summarized in Table 10. Data for single low-dose group in the main study were consistent with data from the preliminary study. Radioactivity in the blood was at detection limits within 24 to 48 hours for all experimental groups. Graphic plots of blood concentration data indicated a biphasic elimination at 8 hours.

TABLE 9. Pharmacokinetic parameters for orally administered [α-^{14}C]XRD-537 BE and [β-^{14}C]XRD-537 BE (1 mg/kg) in rats (Preliminary study)[a]				
Parameter	[α-^{14}C]XRD-537 BE		[β-^{14}C]XRD-537 BE	
	Male	Female	Male	Female
C_{max} (μg eq./mL	1.42/1.84	0.63/0.62	1.54/1.57	0.57/1.0
t_{cmax} (hrs)	2/1	1/1	2/1	1/0.5
$t_{1/2}$ (hrs)	8.3	1.9/1.6	5.1/2.9	-/1.4
AUC (μg eq. · hr · mL^{-1}) 0-12 hrs (0-24 hrs for α-label)	11.5/11.2	2.68/2.08	12.2/9.94	2.69/2.29
AUC (μg eq. · hr · mL^{-1}) 0-∞	11.5/11.3	2.69/2.10	12.3/9.98	2.70/2.31

[a] Data for two rats only
Data taken from pp. 72-74, MRID 45000426.

TABLE 10. Pharmacokinetic parameters for orally administered [α-^{14}C]XRD-537 BE in rats (Main study)						
Parameter	Single low dose (1 mg/kg)		Single high dose (50 mg/kg)		Repeat dose (1 mg/kg)	
	Male	Female	Male	Female	Male	Female
C_{max} (μg eq./mL)	1.30	0.73	76.61	42.60	1.23	0.58
t_{cmax} (hrs)	2	0.5	4	2	2	0.5
$t_{1/2}$ (hrs)	3	3.9	2.7	7.9	3.1	1.4
AUC (μg eq. · hr · mL^{-1}) 0-12 hrs	9.52	3.12	677	318	8.28	2.21
AUC (μg eq. · hr · mL^{-1}) 0-∞	9.56	3.14	679	321	8.32	2.28

Data taken from Tables 5-8 and 19-20, Figures, 5-8 and 19-20, pp. 121-130, and 165-168, MRID 45000427.

C. METABOLITE CHARACTERIZATION STUDIES

1. Plasma

In the preliminary study, the acid metabolite (R-(+)-2-[4-(4-cyano-2-fluorophenoxy) phenoxy]propanoic acid) represented >93% of the plasma radioactivity at 1 to 4 hours after dosing with either the α-^{14}C or β-^{14}C label. No parent compound or other metabolites were detected. There was no appreciable differences in the quantitative or qualitative plasma metabolite profiles between males and females.

Results from the main study also showed that the majority of the radioactivity was associated with the acid metabolite and that no parent compound was present (Table 11). Except for the high dose group where plasma for both males and females was sampled at 9 hours post-dose, difference in sample times made gender comparisons difficult. For the high-dose group at 9 hours, plasma from females (mean of five rats) exhibited somewhat lower average proportions of the acid metabolite and correspondingly higher proportions of other (uncharacterized) metabolites. This variance, however, could be attributed to one female rat (#136) with substantially lower values for the acid metabolite and higher values for the uncharacterized metabolites.

2. Urine

In the preliminary study, neither TLC nor HPLC detected parent compound in the 24-hour urine samples of male and female rats given either [α-^{14}C]XRD-537 BE or [β-^{14}C]XRD-537 BE. Greater than 90% of the urinary radioactivity (equivalent to 85% of the administered dose) was attributed to the acid metabolite, R-(+)-2-[4-(4-cyano-2-fluorophenoxy)phenoxy]propanoic acid, and one unknown metabolite (<1% of urinary radioactivity) was detected in samples from some rats.

In the main study, no parent compound was found in the 0-24 hour urine samples of any of the dose groups. The acid metabolite represented ~71-87% of the urinary radioactivity (~67-79% of the administered dose); neither dose-related nor gender-related differences were exhibited. DP, (4-(2-fluoro-4-cyanophenoxyl)phenol), was detected only in the urine of male rats of the repeat dose group and represented only 0.7% of the urinary radioactivity (0.6% of dose). Uncharacterized metabolites (i.e., radioactivity not eluting in defined peaks following HPLC separation) accounted for ~9-26% of the urinary radioactivity, the lower end of this range being in the repeated dose group. There did not appear to be biologically relevant gender-specific differences in the qualitative or quantitative profiles. These data are summarized in Table 11.

3. Feces

In the preliminary study, neither TLC nor HPLC detected parent compound in the 24-hour fecal samples of male and female rats given either [α-^{14}C]XRD-537 BE or [β-^{14}C]XRD-537 BE. The acid metabolite (~68-77% of fecal radioactivity) was detected in samples from male but not female rats.

The metabolite profile for 0-24 hour fecal samples from the main study experimental groups was similar to that observed in the preliminary study (Table 11). No parent compound was detected and most (~46-75%) of the fecal radioactivity was associated with the acid metabolite. Across treatment groups, this metabolite represented ~0.5-14% of the administered dose with the highest percentage occurring in the repeated dose group. Slightly greater amounts of the acid metabolite were found in the feces of males in all dose groups than females of the respective groups. Metabolite RFa1 was also found and accounted for 6.1-7.7% of the radioactivity in the feces, but represented only 0.1-0.3% of the administered dose. Radioactivity not associated with defined elution peaks following HPLC accounted for 14.7-17.8% and 16.8-35% of the radioactivity in the fecal samples from males and females, respectively. This difference was consistent with the quantitative difference observed for the acid metabolite where seemingly lower amounts were detected in the feces of female rats than for males.

TABLE 11. Metabolite profiles (% of specific matrix radioactivity) in urine, feces, and plasma of rats following oral administration of [α-^{14}C]XRD-537 BE[a]

atrix	Low dose		High dose		Repeated dose	
	Males	Females	Males	Females	Males	Females
Urine						
XRD-537 BE	ND	ND	ND	ND	ND	ND
Acid[b]	80.7	70.9	79.2	76.3	87.2	84.6
DP[c]	ND	ND	ND	ND	0.7	ND
Others[d]	17.6	26.3	19.0	20.4	9.3	14.2
Feces						
XRD-537 BE	ND	ND	ND	ND	ND	ND
Acid	69.1	50.4	74.8	46.3	61.2	68.6
RFa1	7.7	6.2	6.1	7.4	7.0	3.4
Others	17.8	35.0	12.8	37.2	14.7	16.8
Plasma						
XRD-537 BE	ND	ND	ND	ND	ND	ND
Acid	94.2 (6-hr)	78.7 (3-hr)	90.4 (9 hr)	74.8 (9-hr)	94.1 (6-hr)	80.5 (3-hr)
DP	0.3 (6-hr)	ND	0.5 (9-hr)	ND	0.2 (6-hr)	ND
RPa1	0.3 (6-hr)	ND	ND	ND	0.2 (6-hr)	ND
RPa2	ND	ND	ND	ND	ND	ND
Others	3.3 (6-hr)	16.8 (3-hr)	8.0 (9-hr)	21.9 (9-hr)	3.2 (6-hr)	18.0 (3-hr)

[a] 0-24 hr samples except plasma as noted; [b] (R-(+)-2-[4-(4-cyano-2-fluorophenoxy)phenoxy]propanoic acid); [c] 4-(2-fluoro-4-cyanophenoxyl)phenol; [d] no defined elution peak by HPLC
ND: not detected
Data taken from Tables 23-1, 24-1, 25-1, 26-1, 27-1 and 28-1, pp. 178, 181, 184, 187, 190, 193, and 196 of MRID 45000427.

4. Bile

In the preliminary study, neither TLC nor HPLC detected parent compound in the 24-hour bile samples of male and female rats given either [α-^{14}C]XRD-537 BE or [β-^{14}C]XRD-537 BE. The acid metabolite and four unknown metabolites were detected in bile from rats given either label position. The acid metabolites represented the greatest amount of biliary radioactivity (~18-33% for the α-^{14}C label and 61-68% for the β-^{14}C label). For the α-^{14}C label but not the β-^{14}C label, enzymatic hydrolysis of bile increased the amounts of the acid metabolite.

In the main study, the acid metabolite represented 42-48% of the radioactivity detected in the 0-24 hour bile samples from both males and females (Table 12). Label position did not appear to significantly affect, quantitatively or qualitatively, the metabolite profile. Hydrolysis of bile samples with β-glucuronidase/arylsulfatase decreased the radioactivity associated with RBb0 and RBb1 indicating that they were conjugation products.

TABLE 12. Metabolite profiles (% of specific matrix radioactivity) in bile of rats following oral administration of [α-^{14}C]XRD-537 BE or [β-^{14}C]XRD-537BE[a] (1 mg/kg)[a]

Matrix	Males	Females
[α-^{14}C]XRD-537 BE		
XRD-537	ND	ND
Acid[b]	48.0	47.7
DP[c]	ND	ND
RBa0	13.8	8.8
RBa1	25.6	19.2
RBa2	0.5	ND
RBa3	2.2	2.4
Others[d]	8.8	17.0
[β-^{14}C]XRD-537 BE		
XRD-537 BE	ND	ND
Acid[b]	42.8	42.0
DP[c]	0.6	ND
RBb0	15.6	7.7
RBb1	26.4	17.6
RBb2	ND	ND
RBb3	1.9	2.3
Others[d]	10.5	28.6

[a] 0-24 hr samples, non-hydrolyzed; [b] (R-(+)-2-[4-(4-cyano-2-fluorophenoxy)phenoxy]propanoic acid); [c] 4-(2-fluoro-4-cyanophenoxyl)phenol; [d] no defined elution peak by HPLC
ND: not detected
Data taken from Tables 29-1 and 30-1, pp. 196 and 200 of MRID 45000427.

4. Tissues

The main study examined metabolite profiles in the liver and kidneys. No parent compound was detected in either of these organs. Most (~49-84%) of the radioactivity associated with the liver was attributed to the acid metabolite. The metabolite, DP (4-(2-fluoro-4-cyanophenoxyl)phenol), was also detected and accounted for approximately 1-3% of the liver radioactivity regardless of dose. Two uncharacterized metabolites were also found that generally represented <3% of the liver radioactivity. At initial sampling times (2-4 hrs), the acid metabolite generally represented 82-88% of the radioactivity in the kidneys of low-dose (single and repeated) dose groups but only 67-70% in the high-dose group. An additional difference in the kidney metabolite profile of the high-dose group was an absence of the DP metabolite and RKa2 both of which were detected in minor amounts (<2%) in the kidneys of single and repeated low-dose groups. Results of both the preliminary and main studies showed that metabolite profiles for liver and kidney generally paralleled that of blood and bile in that parent compound was not detected and the majority of the radioactivity was associated with the acid metabolite. Metabolite levels in tissues notably decreased by nine hours after dosing.

III. DISCUSSION

A. DISCUSSION

A preliminary study (MRID 45000426) and a main study (MRID 45000427) were conducted to evaluate the metabolism and disposition of XRD-537 BE in male and female Fischer rats. Non-labeled XRD-537 BE (Lot no. AGR 295713, purity >97%) and radiolabeled XRD-537 BE ([α-^{14}C] XRD-537 BE, Lot no. A-903-48 or [β-^{14}C] XRD-537 BE, Lot no. A-903-34a, >97% radiochemical purity for both label positions) was administered orally to groups of five to seven male and female Fischer rats. Dose groups in the main study (MRID 45000427) included single low dose (1 mg/kg), single high dose (50 mg/kg), and 14-day repeated low dose (1 mg/kg) and were used to assess mass balance (absorption, excretion, tissue burdens), pharmacokinetics, biliary excretion, and metabolite profiles. In the preliminary study (MRID 45000426) using the same test articles assessed mass balance, pharmacokinetics, biliary excretion, metabolite profiles in groups of two to four male and female Fisher rats given a single 1 mg/kg dose were studied. Stability and homogeneity of the dose formulation was assessed in a separate study (MRID 45000528).

Mass balance in both the preliminary and main study was acceptable with overall recovery of administered radioactivity ranging from 94-104%. Based upon excretion and tissue burden data, absorption of the test material was shown to be 93-100% and was consistent in both the preliminary and main study, among the various dose groups, and for both label positions. Time-to-maximum plasma concentration (t_{cmax}) was less than 0.5 to 4 hours suggesting rapid absorption regardless of dose.

Urinary excretion was the major route of elimination regardless of dose, label position, or gender. Over a 168-hour period, 84-100% of the administered radioactivity was eliminated via the urine in both the preliminary and main study. Time-course analysis of urinary excretion revealed that approximately 86- 90% of the administered radioactivity was eliminated within 24 hours and that only residual radioactivity (<1%) was appearing after 48 hours. The feces represented a minor route of excretion (<5% of the administered dose) over the 168-hour time period. There was no evidence for expired air as a route of elimination. The relatively rapid urinary elimination implied equally rapid absorption of the test article following oral administration.

There was considerable individual variability in biliary excretion. This, and the limited number of experimental animals, made it difficult to assess possible gender-related or label position-related differences in biliary elimination. Results of the preliminary and main studies showed that over a 24-hour period, biliary elimination accounted for 1.7 % and 20.1% of the administered dose in males and females, respectively, in the low-dose [α-^{14}C]XRD-537 BE group. For the groups receiving a single low dose of [β-^{14}C]XRD-537 BE, 24-hour biliary elimination accounted for 17.0% (males) and 11.6% (females) of the administered dose.

Tissue distribution studies indicated that the greatest radioactivity over 24 hours occurred in the liver, kidneys, plasma, whole blood, heart, lung, and stomach. The highest tissue

concentrations (2-hr liver and kidney) represented 15-19% of the administered dose while most tissue levels accounted for <1% of the administered dose. Tissue/organ burdens appeared to be somewhat less in females while concentrations in gastrointestinal organs were somewhat greater suggesting decreased absorption. Not all the concentration values for tissues/organs reflected the 50-fold difference in dose, thereby suggesting dose-related variance in absorption/distribution/excretion processes. Consistent with the rapid excretion of test article-related radioactivity, tissue/organ levels of radioactivity notably declined to near detection limits at 24 hours. There was no evidence of tissue/organ sequestration in either the low-, high-dose, or repeat-dose group. Clearance of radioactivity from the tissues following repeated dosing was also similar to that for the single low-dose group in that levels were near or below detection limits at 24 hours.

Blood concentration time-course data from both the preliminary and main studies showed a biphasic pattern for both [α-^{14}C]XRD-537 BE and [β-^{14}C]XRD-537 BE. There were no substantial differences in pharmacokinetic indices (C_{max}, t_{cmax}, $t_{1/2}$, AUC) between the two label positions based upon data from the preliminary study. Time-to-maximum plasma concentration (t_{cmax} of 0.5 to 4 hrs) reflected the relatively rapid absorption; longer t_{cmax} values were expectedly associated with the higher dose. Similarly, elimination half-times ($t_{1/2}$) also reflected the rapid absorption/distribution/excretion of the test article. There was evidence for gender-related differences, however, with females exhibiting somewhat shorter t_{cmax} and lower C_{max} values suggestive of saturated absorption processes. This was also reflected in two- to four-fold lower AUC values for females.

Metabolite characterization revealed that the acid metabolite (R-(+)-2-[4-(4-cyano-2-fluorophenoxy)phenoxy]propanoic acid) was the most prominent fraction in plasma representing ~90-94% of the dose in males and ~75-81% in females regardless of dose. Results of the preliminary study also showed that the acid metabolite represented >93% of the administered radioactivity at 1 to 4 hours after dosing with either the α-^{14}C or β-^{14}C label. No parent compound or other metabolites were detected. There was no appreciable differences in the quantitative or qualitative plasma metabolite profiles between males and females. Urinary and fecal metabolite profiles also showed that the acid metabolite was the most prevalent biotransformation product accounting for approximately 46-75% (feces) and 71-87% (urine) of the administered. Gender-related differences were observed with somewhat lesser amounts being detected for females than for males. This difference was accounted for by somewhat greater levels of uncharacterized components in the urine and feces of females. A proposed metabolism pathway was provided that was consistent with the findings of the studies (Figure 1).

These rat metabolism/kinetics studies are collectively **Acceptable/Guideline** and satisfy the requirements for a Metabolism and Pharmacokinetics Study [OPPTS 870.7485 (§85-1)].

B. STUDY DEFICIENCIES

The inordinately low values for one of the female rats in the [α-^{14}C]XRD-537 BE group appeared to be indicative of a dysfunctional bile duct cannula but this was not possible to determine with certainty in the absence of bile flow data. The considerable individual

variability in biliary excretion made difficult a valid assessment of gender-related or label position-related differences in biliary elimination. There were no deficiencies that compromised the validity of the study protocols, results or conclusions.

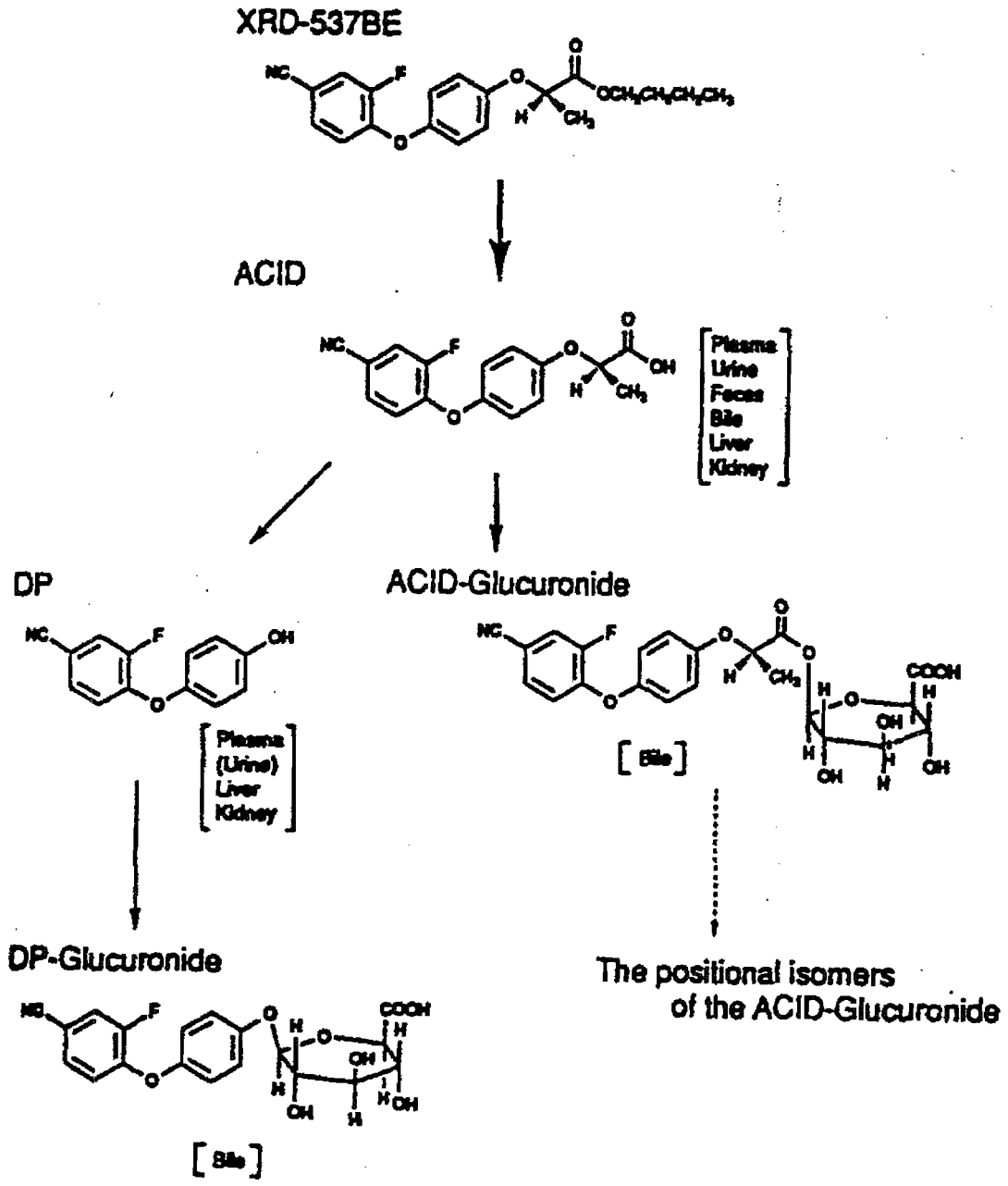


Figure 1. Proposed metabolism pathway for XRD-537BE in rats.
Figure taken from p. 107, MRID 45000427.

DATA EVALUATION RECORD

CYHALOFOP BUTYL
(DE-537 N-BUTYL ESTER)

STUDY TYPE: DERMAL PENETRATION – RAT
[OPPTS 870.7600 (§85-3)]
MRID 45000505

Prepared for
Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
1921 Jefferson Davis Highway
Arlington, VA 22202

Prepared by

Chemical Hazard Evaluation Group
Toxicology and Risk Analysis Section
Life Sciences Division
Oak Ridge National Laboratory
Oak Ridge, TN 37831
Task Order No. 01-81-MM

Primary Reviewer: Robert A.Young, Ph.D., D.A.B.T.	Signature: Date:	Robert A. Young FEB 0 9 2001
Secondary Reviewer: H. Tim Borges, Ph.D., MT (ASCP), D.A.B.T.	Signature: Date:	H T Borges FEB 0 9 2001
Robert H. Ross, M.S., Group Leader	Signature: Date:	Robert H. Ross FEB 0 9 2001
Quality Assurance: Lee Ann Wilson, M.A.	Signature: Date:	L. A. Wilson FEB 0 9 2001

Disclaimer

This review may have been altered subsequent to the contractor's signatures above.

Oak Ridge National Laboratory, managed by UTBattelle, LLC, for the U. S. Dept. of Energy under contract DEAC0500OR22725.

CYHALOFOP BUTYL (DE-537 N-BUTYL ESTER) **Dermal Penetration Study [OPPTS 870.7600 (§85-3)]**

EPA Reviewer: John Whalan, Date 5-4-01
Registration Action Branch 2 (7509C)
EPA Work Assignment Manager: SanYvette Williams-Foy, D.V.M. _____, Date 5/4/01
Registration Action Branch 2 (7509C)
TXR No.: 0050348

DATA EVALUATION RECORD

STUDY TYPE: Dermal penetration - Rat [§85-3)]

DP BARCODE: D268553
P.C. CODE: 082583

SUBMISSION CODE: S
TOX. CHEM. NO.: not provided

TEST MATERIAL (PURITY): DE-537 n-butyl ester (chemical purity 97.1%)

SYNONYMS: DE-537 n-butyl ester (BE); XRD 537 BE; Cyhalofop-butyl

CITATION: Dryzga, M.D., Domoradzki, J.Y., Hansen, S.C. (1998) DE-537 N-Butyl Ester: Dermal Absorption of 14C-DE-537-Butyl Ester in Male Fischer 344 Rats Following Exposure to Undiluted EF-1218 and a Spray Solution. Lab. Project Study No. 981090. Health & Environmental Research Laboratories, The Dow Chemical Company, Midland, MI 48674. MRID 45000505. Unpublished. 12 November 1998.

SPONSOR: Dow AgroSciences (DAS) LLC, Letcombe Regis, England..

EXECUTIVE SUMMARY: In a dermal penetration study in rats (MRID 45000505), groups of four male Fischer 344 rats were given ^{14}C-labeled DE-537 n-butyl ester (Lot No. 1374, radiochemical purity >99.7%, sp. act. 30.1 mCi/mmole) and nonlabeled DE-537 n-butyl ester (Lot. No. DECO-26-42T, chemical purity >97.1%) dermally in two formulations (spray and with EF-1218 vehicle) at concentrations of 0.005, 1.0, or 1.8 mg/cm^2 for 24-hours. Absorption, excretion and tissue burdens were assessed 24, 48, and 72 hours postdose as well as potential for adherence to the skin application appliance components.

No signs of test article-related toxicity were reported. Recovery of radioactivity was 93-101% and did not vary significantly among the various treatment groups. Based upon radioactivity inventory from urine, feces, tissues/carcass, and cage wash, absorption was approximately 25-34% for the spray formulation, 11-16% for the EF-1218 formulation, and 16% for the EF-1218 formulation with Teflon appliance. Very little absorption occurred after 48 hours. Although a greater percent of the administered dose was absorbed in groups receiving the diluted spray formulation, actual amount absorbed was greater for the groups given the EF-1218 formulation. Blood concentrations at 24 hours after exposure, however, suggested that dermal absorption processes may have been saturated for the EF-1218 groups. Adsorption of test article to the appliance components was found to be relatively inconsequential in affecting dermal penetration. The use of a Teflon frame and covering, and a less absorbent bonding agent resulted in greater availability (92% vs 60-70% for the normal appliance components) of test material for absorption

but similar total absorption (~16% for the Teflon appliance vs ~14-16% for the conventional appliance). Most of the unabsorbed radioactivity was associated with the appliance components.

Radioactivity in the blood, liver, kidneys, remote skin, and carcass declined rapidly from 24 to 48 hours which was consistent with the absorption/excretion time course. More than 85% of urinary radioactivity excretion occurred within 48 hours. Less than 1% of the administered dose was excreted in the feces. The occurrence of radioactivity in the liver and kidneys was consistent with metabolism to water soluble metabolites and subsequent urinary excretion.

Under the conditions of this study, 24-hour dermal application of two formulations containing radiolabeled DE-537 n-butyl ester resulted in moderate dermal absorption in the rat, followed by systemic distribution and excretion of administered radioactivity via the urine.

This study is **Acceptable/Non-Guideline.** Because only one exposure duration was used (i.e., 24 hours), the study does not satisfy (§85-3) requirements for a dermal penetration study which dictate six exposure durations (i.e., 0.5, 1, 2, 4, 10, and 24 hrs).

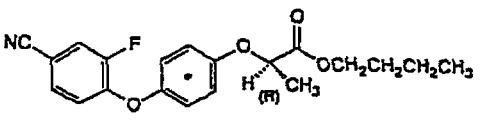
COMPLIANCE: Signed and dated GLP and OECD Principles of Good Laboratory Practice (p. 3), Quality Assurance p.4), and Data Confidentiality (p. 2) statements were provided. A Flagging statement was not included.

I. MATERIALS AND METHODS

A. MATERIALS

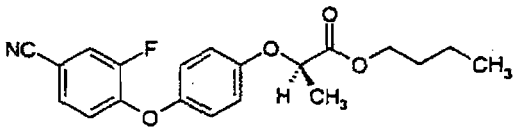
1. Test compounds

Radiolabeled: DE-537-α-Ph-UL-^{14}C-n-butyl ester
Radiochemical purity: >99.7%
Specific activity: 30.1 mCi/mmole
Batch/Lot No.: #1374 (Specialty Synthesis Databook ref. # F0449-29)
Description: white crystalline solid
Structure:



• : ^{14}C

Non-radiolabeled:
Chemical purity: >97.1%
Batch/Lot No.: #DECO-26-42T (AGR295713)
Description: white crystalline solid
Structure:



2. Vehicle

The test material (radiolabeled and non-labeled DE-537 n-butyl ester) were added to EF-1218 (blank formulation, Lot # C0575-8-1).

3. Test animals

Species: Rat (male)
Strain: Fischer 344
Age and weight at study initiation: ~10 weeks;
Source: Charles River Laboratories Inc. (Raleigh, NC)
Housing: Two rats per cage during acclimation. Acclimated for two days to Roth metabolism cages prior to test period.
Diet: Purina Certified Rodent Lab Diet #5002 (Purina Mills, Inc. St. Louis, MO) provided *ad libitum.*
Water: tap water (analyzed by municipal water department) was provided *ad libitum.*
Environmental conditions:
Temperature: not provided
Humidity: not provided
Air changes: not provided
Photoperiod: 12 hr light/dark
Acclimation: Rats were acclimated at least one week.

4. Preparation of dosing solutions

The dosing solutions were prepared by mixing appropriate amounts of ^{14}C-DE-537 n-butyl ester and non labeled DE-537 n-butyl ester with EF-1218 (Clincher EC with which DE-537 n-butyl ester is normally formulated) to obtain a concentration of 200 mg/mL. A spray solution was also formulated using labeled and non-labeled DE-537 n-butyl ester, and 0.1% (w/w) polyglycol 26-2N (Lot no. 98RM816) to obtain a test material concentration of 0.4 mg/mL.

Results –
Homogeneity – Data regarding homogeneity of dose solutions was not provided although it was noted that aliquots of the dosing solutions were analyzed for radioactivity.

Stability – Stability was confirmed by high performance liquid chromatography (HPLC)

Dose confirmation – Results of analyses were provided showing that radioactivity was 96-109% of target and test article concentrations were 84-108% of target.

B. STUDY DESIGN AND METHODS

1. Group arrangements

Groups of four male rats were organized into the test groups shown in Table 1. No information was provided regarding assignment of individual rats to the various groups.

TABLE 1. Study design for dermal absorption of

Experimental Group	Dose	No./Sex	Comments
Group 1	0.005 mg/cm^2	4/M	Low dose spray (0.4 mg/ml); 24 hr collection/sacrifice time
Group 2	0.005 mg/cm^2	4/M	Low dose spray (0.4 mg/ml)
Group 3	0.005 mg/cm^2	4/M	Low dose spray (0.4 mg/ml)
Group 4	1.8 mg/cm^2	4/M	Single dermal high dose; 24-hr collection/sacrifice time
Group 5	1.8 mg/cm^2	4/M	Single dermal high dose; 24 and 48-hr collection times
Group 6	1.8 mg/cm^2	4/M	Single dermal high dose; 24, 48, and 72-hr collection times
Group 7	21 mg/12 cm^2	4/M	Single dermal; 24 hr collection/sacrifice time; for assessment of absorption of test article by appliance components (teflon frame used to prevent absorption of test material to appliance components)

Information taken from pp.19-20 and Table 1 (pp. 33-34), MRID 45000505.

2. Dosing and sample collection

The test material was applied to a 12 cm^2 area on the dorsal surface of rats. The application sites were shaved and Stomahesive dose frames attached with Skin-Bond adhesive 24 hours prior to dosing (Group 7 was fitted with a Teflon frame and Permabond Industrial Grade 910 adhesive). Rats were anesthetized with methoxyflurane for site preparation and dosing. The test materials (both concentrated formulation and dilute spray) were applied with a glass syringe equipped with round-tipped feeding needle. Application volume was ~10 μL/cm^2. The syringe and needle were weighed before and after dosing. The dosing areas were then protected with a Teflon film and Vetrap bandage. Immediately after dosing the rats were placed in Roth metabolism cages.

Expired CO_2/volatile organics - Expired air and volatile organics were not collected as previous studies indicated that these matrices did not contain elimination products.

Blood - Blood was collected from rats in Groups 1, 4 and 7 at the 24-hour termination time.

Urine - Urine was collected over dry-ice at 24-hour intervals. Samples were weighed and retained for analysis.

Feces - Feces were collected over dry ice at 24-hour intervals and retained for analysis.

Tissues - In addition to dose-site and remote skin samples, the liver and kidneys were collected at termination for organ-specific quantification of radioactivity.

Carcass/skin - The carcasses were retained for analysis of radioactivity at termination. Both dose site and remote (skin other than at dose site) skin samples were retained for analysis.

Cage wash - Cage wash was collected concurrently with urine samples.

Unabsorbed dose (non-biological samples) - At specified times (24 hours for Groups 1, 4, and 7; 24 and 48 hours for Groups 2 and 5; 24, 48, and 72 hours for Groups 3 and 6), the bandage material was removed and saved for analysis. The dose site was washed with soap and water, rinsed several times, repeatedly stripped with adhesive tape and all materials retained for radioactivity determination. All appliance components and extracts, and skin washes were retained for radioactivity analysis.

4. Sample preparation/analysis

Blood - Blood samples were solubilized (no details provided) and radioactivity determined by liquid scintillation counting (LSC). Blood samples were also analyzed for DE-537 n-butyl ester and DE-537 by high-performance liquid chromatography (HPLC) and gas chromatography/electron ionization/mass spectrophotometry (GC/EI/MS) analysis. Blood analysis included a control sample from an untreated rat.

Urine - Weighed aliquots were analyzed for radioactivity by LSC. No details were provided regarding sample preparation.

Feces - Fecal samples were homogenized in water (25% feces:water, w/w) and radioactivity determined in weighed aliquots following solubilization (no further details provided).

Tissues - Tissue samples were homogenized and solubilized prior to LSC analysis.

Carcass/skin - Carcasses were homogenized and solubilized, and skin samples were solubilized prior to LSC analysis.

Cage wash - Cage wash samples were analyzed by LSC.

Unabsorbed dose (non-biological samples) - All appliance components and extracts, and skin washes were analyzed by LSC. No details were provided.

5. Analytical techniques

Liquid Scintillation Counting LSC - LSC was performed using a Beckman LS 3801 or LS 1801. Counts per minute were quench and background corrected, and converted to DPM. A standard was used to monitor instrument performance. Samples with DPMs of less than twice background were ignored.

HPLC- HPLC was used for dose confirmation. The system included a Waters C_{18} Bondpak 3.9 mm x 300 mm column operated at a flow rate of 1 mL/minute and a mobile phase of water/1% acetic acid (A) and acetonitrile/1% acetic acid (B). A gradient solvent flow (95%A - 5% B to 5%A - 95%B over 30 minutes and returning to 95/5 A/B at 45 minutes) was used. Detection was at 254 nm. For blood sample analysis and dose solution stability, an Hitachi 6200A with the above column (guard column attached) and mobile phases were employed. Gradient flow (1 mL/min) of 95%A/5%B to 5%A/95%B over 30 minutes was used. A radioflow detector (Berthold LB 509) and Beckman LSC counter (LS6000) were also used.

(GC/EI/MS) gas chromatography/electron ionization/mass spectrophotometry analysis - Dose solution concentrations were determined with weighed aliquots of samples and standards of ~8-12 μg/mL. A J&W DB-5MS 30 m x 0.25 mm column was used. Operating parameters were reported.

6. Statistics

Statistical analysis was limited to descriptive statistics (variability about the mean).

II. RESULTS

A. RADIOACTIVITY INVENTORY

Recovery of administered radioactivity was 100-101% for the spray test article (Groups 1-3), 93-96% for the EF-1218/DE-537 n-butyl ester formulation (Groups 4-6), and 90% for the special (Teflon frame) Group 7 (Table 2).

TABLE 2. Recovery (% of administered dose) of Radioactivity in Rats Following Dermal Application of DE-537-Ph-UL-^{14}C-n-butyl ester*							
Matrix	Spray Groups			Formulation Groups			Special Group[a]
	Group 1 (24 hr)	Group 2 (48 hr)	Group 3 (72 hr)	Group 4 (24 hr)	Group 5 (48 hr)	Group 6 (72 hr)	Group 7 (24 hr)
Dosed skin	26.93±9.75	25.67±4.43	19.12±3.81	11.76±3.95	7.44±3.78	9.73±3.54	3.14±2.06
Urine	18.35±9.10	31.20±9.30	29.78±4.70	6.34±1.41	14.11±0.79	12.97±2.62	12.62±2.20
Feces	0.38±0.09	0.52±0.14	0.94±0.38	0.22±0.29	0.40±0.23	0.35±0.12	0.23±0.09
Tissues	5.08±2.93	1.03±0.78	0.53±0.36	2.49±0.34	0.85±0.28	0.26±0.07	2.50±0.56
Appliance	16.35±2.39	16.04±2.22	16.87±1.39	52.08±5.48	47.35±6.23	44.01±10.20	49.30±4.08
Removed dose	32.80±9.08	25.36±9.296	33.04±4.00	22.03±8.88	26.00±8.38	26.06±5.76	21.78±5.08
Final cage wash	1.27±0.35	0.92±0.52	<0.49±>0.44	1.53±0.92	0.35±0.12	0.25±0.07	0.32±0.18
Total	101.173.21	100.73±1.90	100.77±1.90	96.45±2.97	96.50±0.90	93.61±2.75	89.89±1.55

* Values are a mean of four rats.
[a] Teflon appliance
Data taken from Tables 12-14, pp. 45-47, MRID 45000505.

B. ABSORPTION

Absorption of dermally applied test material assessed from radioactivity recovered in urine, feces, tissues, and final cage wash ranged from ~25-34% for the spray test article (Groups 1-3), ~11-16% for the EF-1218/DE-537 n-butyl ester formulation (Groups 4-6), and ~16% for the special (Teflon frame) Group 7 (Table 3). Most (60-94%) of the absorbed radioactivity was excreted in the urine. Maximum absorption appeared to be achieved within 48 hours regardless of formulation.

TABLE 3. Recovery (% of administered dose) of Radioactivity in Rats Following Dermal Application of DE-537-Ph-UL-^{14}C-n-butyl ester*							
Matrix	Spray Groups			Formulation Groups			Special Group
	Group 1 (24 hr)	Group 2 (48 hr)	Group 3 (72 hr)	Group 4 (24 hr)	Group 5 (48 hr)	Group 6 (72 hr)	Group 7 (24 hr)
Urine	18.35±9.10	31.20±9.30	29.78±4.70	6.34±1.41	14.11±0.79	12.97±2.62	12.62±2.20
Feces	0.38±0.09	0.52±0.14	0.94±0.38	0.22±0.29	0.40±0.23	0.35±0.12	0.23±0.09
Tissues	5.08±2.93	1.03±0.78	0.53±0.36	2.49±0.34	0.85±0.28	0.26±0.07	2.50±0.56
Cage wash	1.27±0.35	0.92±0.52	0.49±0.44	1.53±0.92	0.35±0.12	0.25±0.07	0.32±0.18
Total	25.08±6.28	33.67±9.40	31.74±4.50	10.58±2.35	15.70±0.90	13.81±2.62	15.67±2.75

* Values are each a mean of four rats.
Data taken from Tables 15-17, pp. 48-50, MRID 45000505.

C. TISSUE DISTRIBUTION

Tissue distribution of test article-related radioactivity expressed as percent of administered dose is summarized in Table 4. Tissue radioactivity (including carcass) accounted for 5% or less of the administered dose. Over the 72-hour period, blood concentrations declined to near detection limits which was consistent with observations on urinary elimination. When expressed as μg equivalents per gram tissue, the tissue levels were quite low: <0.05 μg eq/g tissue for the spray group, <8 μg eq/g tissue for the EF-1218 formulation group, and up to 11 μg eq/g tissue for the special (Teflon appliance) group.

TABLE 4. Tissue Distribution (% of administered dose) of Radioactivity in Rats Following Dermal Application of DE-537-Ph-UL-^{14}C-n-butyl ester*

Tissue	Spray Groups			Formulation Groups			Special Group
	Group 1 (24 hr)	Group 2 (48 hr)	Group 3 (72 hr)	Group 4 (24 hr)	Group 5 (48 hr)	Group 6 (72 hr)	Group 7 (24 hr)
Blood	0.26±0.04	0.06±0.02	0.01±0.00	0.13±0.04	0.03±0.01	0.01±0.00	0.19±0.08
Carcass	1.96±1.33	NQ	NQ	1.32±0.33	0.25±0.07	NQ	0.95±0.26
Kidneys	0.13±0.01	0.07±0.03	0.01±0.00	0.06±0.01	0.03±0.01	0.01±0.00	0.10± 0.03
Liver	0.54±0.10	0.16±0.06	NQ	0.24±0.04	0.09±0.04	0.03±0.01	0.39±0.15
Remote skin	2.19±1.64	0.45±0.22	0.49±0.34	0.74±0.44	0.45±0.21	0.22±0.08	0.87±0.09
Total	5.08±2.93	1.03±0.78	0.53±0.36	2.49±0.34	0.85±0.28	0.26±0.07	2.50±0.56

* Values are each a mean of four rats; NQ: non-quantifiable
Data taken from Tables 26-28, pp. 59-61, MRID 45000505.

D. EXCRETION

As shown in Table 2, the majority of the absorbed radioactivity for all groups was excreted in the urine. Examination of individual animal data revealed considerable variability in the 24-hour spray group (Group 1) but no appreciable variability was observed in the remaining groups. Fecal elimination was minimal, accounting for less than 1% of the administered dose. Cumulative urinary excretion (data not shown) revealed that >85% of urinary radioactivity occurred within 48 hours.

III. DISCUSSION

A. DISCUSSION

In a dermal penetration study in rats (MRID 45000505), groups of four male Fischer 344 rats were given ^{14}C-labeled DE-537 n-butyl ester (Lot No. 1374, radiochemical purity >99.7%, sp. act. 30.1 mCi/mmole) and nonlabeled DE-537 n-butyl ester (Lot. No. DECO-26-42T, chemical purity >97.1%) dermally in two formulations (spray and with ES-1218 vehicle) at concentrations of 0.005, 1.0, or 1.8 mg/cm^2 for 24 hours. Absorption,

excretion and tissue burdens were assessed at 24, 48, and 72 hours as well as potential for adherence to the skin application appliance components.

The test animals showed no significant signs of test article-related toxicity. Recovery of radioactivity was an acceptable 93-101% and did not vary significantly among the various treatment groups. Based upon radioactivity inventory from urine, feces, tissues/carcass, and cage wash, absorption was approximately 25 to 34% for the spray formulation (Groups 1-3), 11- to 16% for the EF-1218 formulation (Groups 4-6), and 16% for the EF-1218 formulation with Teflon appliance (Group 7). Very little absorption occurred after 48 hours. Although a greater percent of the administered dose was absorbed in groups receiving the diluted spray formulation, actual amount absorbed was greater for the groups given the EF-1218 formulation. Blood concentrations at 24 hours, however, suggested that dermal absorption processes may have been saturated for the EF-1218 groups. Adsorption of test article to the appliance components was found to be relatively inconsequential in affecting dermal penetration. The use of a Teflon frame and covering, and a less absorbent bonding agent resulted in greater availability (92% vs 60-70% for the normal appliance components) of test material for absorption but similar total absorption (~16% for the Teflon appliance vs ~14-16% for the conventional appliance; see Table 2 of this Data Evaluation Record). Most of the unabsorbed radioactivity was associated with the appliance components.

Radioactivity in the blood, liver, kidneys, remote skin, and carcass declined rapidly from 24 to 48 hours which was consistent with the absorption/excretion time course. The occurrence of radioactivity in the liver and kidneys was consistent with metabolism to water soluble metabolites and subsequent urinary excretion.

Under the conditions of this study, both the dilute spray and the EF-1218 formulation of DE-537 n-butyl ester underwent moderate dermal absorption in the rat, followed by systemic distribution and excretion via the urine.

This study is **Acceptable/Non-Guideline**. Because only one exposure duration was used (i.e., 24 hours), the study does not satisfy (§85-3) requirements for a dermal penetration study which dictate six exposure durations (i.e., 0.5, 1, 2, 4, 10, and 24 hrs).

B. STUDY DEFICIENCIES

There were no deficiencies that compromised the validity of the study conduct or its results. However, the study did not utilize log interval doses or multiple exposure durations as specified for an OPP §85-3 Dermal Penetration Study.

DATA EVALUATION RECORD

CYHALOFOP BUTYL

Study Type: HEPATOCELLULAR PROLIFERATION – RAT [NON-GUIDELINE]
MRID 45000414

Prepared for

Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
1921 Jefferson Davis Highway
Arlington, VA 22202

Prepared by

Chemical Hazard Evaluation Group
Toxicology and Risk Analysis Section
Life Sciences Division
Oak Ridge National Laboratory
Oak Ridge, TN 37831
Task Order No. 01-81NN

Primary Reviewer: H. T. Borges, MT(ASCP),Ph.D., D.A.B.T.	Signature:	H.T. Borges
	Date:	JAN 0 5 2001
Secondary Reviewers: Cheryl Bast, Ph.D., D.A.B.T.	Signature:	Cheryl P Bast
	Date:	JAN 0 5 2001
Robert H. Ross, M.S., Group Leader	Signature:	Robert H. Ross
	Date:	JAN 0 5 2001
Quality Assurance: Lee Ann Wilson, M.A.	Signature:	L. A. Wilson
	Date:	JAN 0 5 2001

Disclaimer

This review may have been altered subsequent to the contractor's signatures above.

Oak Ridge National Laboratory, managed by UT-Battelle, LLC, for the U.S. Dept. of Energy under contract DE-AC05-00OR22725

CYHALOFOP BUTYL

Hepatocellular Proliferation, Non-Guideline

EPA Reviewer: John Whalan
Registration Action Branch 2 (7509C)
EPA Work Assignment Manager: S. Williams-Foy, Ph.D.
Registration Action Branch 1 (7509C)
TXR No: 0050348

John Whalan, Date 5-18-01
, Date 5/31/01

DATA EVALUATION RECORD

STUDY TYPE: Hepatocellular Proliferation - Rat (Non-guideline)

DP BARCODE: D268553
P.C. CODE: 082583

SUBMISSION CODE: S
TOX. CHEM. NO.: None

TEST MATERIAL (PURITY): XRD-537nBu (purity 98.8%)

SYNONYMS: Cyhalofop butyl, AGR 276541, R-(+)-n-butyl 2-[4-(2-fluoro-4-cyanophenoxy) phenoxy]propanoate

CITATION: Lomax, L.G., Redmond, J.M., Haut, K.T., Brzak, K.A. (1991). XRD-537nBu: Hepatocellular proliferation study in male Sprague-Dawley rats. The Toxicology Research Laboratory, Health and Environmental Sciences, The Dow Chemical Co., Midland, MI 48674. Laboratory Project ID: DR-0298-8876-008. March 4, 1991. MRID 45000414. Unpublished.

SPONSOR: DowElanco, 9002 Purdue Road, Indianapolis, IN 46268-1189

EXECUTIVE SUMMARY: In a nonguideline hepatocellular proliferation study (MRID 45000414) done to investigate the mechanism of hepatomegaly observed in earlier subchronic studies, groups of 20 male Sprague-Dawley rats were given XRD-537nBu (purity 98.8%, Lot. No. AGR 276541) in the diet at concentrations of 0.0, 3.0, 25, 100, or 400 mg/kg/day. One week before scheduled sacrifice at 1, 2, 4, and 13 weeks, an ALZET osmotic pump was implanted subcutaneously to deliver 10 μL BrdU/hour in 5 rats/group. BrdU is a DNA stain used to quantify hepatocellular proliferation. At sacrifice, the animals were weighed and the liver and duodenum removed, weighed, and prepared for histopathological examination.

Treatment with the test material did not induce premature deaths, clinical signs of toxicity, or effects on body weight. Within one week, however, the test material did induce a dose-related increase in absolute liver weight and liver to body weight in rats fed ≥25 mg/kg/day. The increase was sustained and continued to rise slightly through the remainder of the study. The number of BrdU-labeled nuclei revealed by histochemical staining was dramatically increased in a dose-dependent manner after 7 days of treatment in rats fed ≥25 mg/kg/day test material. The increase was not sustained, and by 14 days was not biologically different from control animals. These results are consistent with an initial dramatic increase in DNA synthesis during the first week of treatment followed by hepatocellular hypertrophy at subsequent observations as an explanation for the enlarged livers observed in XRD-537nBu-treated rats.

This study is considered **Acceptable/Non-Guideline** for the determination of hepatocellular proliferation in the rat.

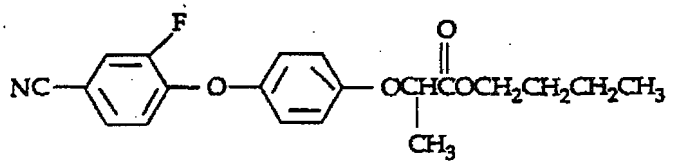
COMPLIANCE: Signed and dated Good Laboratory Practice, Quality Assurance, and Data Confidentiality statements were included with the study. Flagging statements were not included.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test compound: XRD-537nBu

   Lot No.: AGR 276541
   Purity: 98.8%
   Description: solid
   CAS No.: 122008-85-9
   Molecular weight: 357.38 g/mol
   Molecular formula: $C_{20}H_{20}FNO_4$
   Structure:



2. Vehicle

   None

3. Test animals

   Species: Rat
   Strain: Sprague-Dawley
   Sex: male
   Age and weight at study initiation: ~6 weeks and ~285 g
   Source: Charles River Breeding Laboratory, Portage, MI
   Housing: individually in suspended metal cages
   Food: Purina Certified Chow, #5002, *ad libitum*
   Water: tap water, *ad libitum*
   Environmental conditions: not reported
   Acclimation period: ~10 days

4. Preparation of diets

   A 1-3% premix diet was prepared by dissolving the test material in a small amount of acetone. Test diets were prepared weekly by adding the appropriate amount of pre-mix with basal diet according to specifications described in earlier subchronic and

chronic toxicity studies. Newly prepared diets were based on body weights and food consumption data for the previous week. Dietary analyses to ensure target concentrations were done during weeks 1, 5, 9, and 12.

Results –
Homogeneity: Homogeneity of test material in the diets was confirmed in another study (MRID 45000528).

Stability: Earlier studies had shown the test diets to be stable for at least 32 days (MRID 45000413). In this study, diets were prepared weekly.

Dose confirmation: Actual concentrations of test material in the various diets averaged 100% of nominal with a CV of 0.8%.

B. STUDY DESIGN AND METHODS

1. In life dates

Not reported, but after May 2, 1990 and before March 4, 1991.

2. Animal assignment

One hundred male rats were divided into five treatment groups of 20 rats each that received diets containing 0.0, 3.0, 25, 100, or 400 mg test material/kg/day.

3. Dose selection rationale

In earlier subchronic studies, the liver was shown to be the target organ; having developed hypertrophy and focal necrosis within four weeks of treatment in male rats fed diets containing 25, 400, or 1600 mg test material/kg/day and female rats fed 800 or 1600 mg test material/kg/day. After 13 weeks of treatment, hepatocellular hypertrophy was still present in male rats fed 25, 100, or 400 mg test material/kg/day and in female rats fed 100, 400, or 800 mg test material/day. Focal necrosis was present only in 100 mg/kg/day male and female rats. The present study was done to investigate the cause of the hepatocellular proliferation induced.

4. Statistics

The mean and standard deviation were calculated for food consumption. All other parameters were checked for equality of variance by Bartlett's test. If parametric, the data were subjected to analysis of variance with significant differences from control determined by Dunnett's test ($p \leq 0.05$).

C. METHODS

1. Animal observations

All rats were observed at least once daily for treatment-related effects and twice daily for moribundity and death.

2. Body weight and food consumption

Body weights and food consumption were recorded weekly. Animals with implanted pumps were excluded from statistical analyses.

3. Animal preparation

On study day 1, ALZET osmotic pumps (Alza Corp., Palo Alto, Ca) containing 2 mL of 20 mg/mL BrdU (5-bromo-2'-deoxyuridine) and with a flow rate of 10 μL/hour, were subcutaneously implanted into a pocket created along the dorsal midline of 5 rats/group. The pumps remained in place for seven days, after which the rats were sacrificed. This process was repeated on an additional 5 rats/group on study days 8, 23, and 86. BrdU is a DNA stain used to quantify hepatocellular proliferation.

4. Sacrifice and pathology

After 7 days of BrdU osmotic infusion, the rats were killed, the pumps removed, the animals necropsied, and the liver and duodenum removed, weighed, and placed into phosphate-buffered formalin. Following fixation, sections of the left lateral liver lobe and duodenum were embedded in paraffin. The tissues were cut into duplicate 5 μm sections and mounted onto glass slides. One slide was stained by hematoxylin and eosin while the other was immunohistochemically stained for BrdU-labeled DNA. The percent of labeled hepatocytes was determined after counting of 1000 cells. The duodenal sections served as positive BrdU-labeled controls.

II. RESULTS

A. OBSERVATIONS

None of the rats died and no clinical signs of toxicity were observed.

B. BODY WEIGHT AND FOOD CONSUMPTION

No treatment-related effects were found on body weight or food consumption.

C. ORGAN WEIGHT AND PATHOLOGY

1. Liver weight

As shown in Table 1, treatment with ≥25 mg/kg test material increased both the absolute and relative liver weights of male rats within seven days. This effect on the liver persisted and intensified through the subsequent 14 and 28 days in the 25, 100, and 400 mg/kg/day groups before moderating slightly by day 91 of the study.

Table 1. Absolute and relative liver weights of male rats fed diets containing XRD-537nBu up to 91 days[a]

Dose (mg/kg/day)	Absolute (g)				Relative to body weight (g%)			
	Group A (7 d)	Group B (14 d)	Group C (28 d)	Group D (91 d)	Group A (7 d)	Group B (14 d)	Group C (28 d)	Group D (91 d)
0	9.255	9.862	11.168	12.745	3.204	2.966	2.869	2.552
3	9.789	10.757	11.646	13.307	3.321	3.268	2.919	2.484
25	10.597*	11.620	13.984	14.402	3.661*	3.597	3.568*	2.950*
100	12.937*	13.406*	15.892*	18.667*	4.335*	4.188*	4.222*	3.396*
400	12.327*	14.684*	18.165*	20.110*	4.411*	4.717*	4.826*	4.231*

Data from Tables 12-15, pp. 36-39, MRID 45000414
*p≤0.05
[a]Rats were sacrificed after 7, 14, 28, and 91 days of treatment for Groups A-D, respectively.

2. Gross liver pathology

Within one week of treatment, grossly observable hepatomegaly was present in most rats treated with 400 mg/kg/day test material. By the second week and through the remainder of the study, hepatomegaly was grossly observable in all rats in the 100 and 400 mg/kg/day groups. Pale foci were visible on one or more liver lobes of a 400 mg/kg/day animal on day 28 and by day 91 visible in one or more rats in the 25-400 mg/kg/day groups.

3. Liver histopathology

All rats treated with ≥25 mg/kg/day test material developed hepatocellular hypertrophy within 7 days of treatment that persisted through the remainder of the study. Typically, the cytoplasm of the hepatocytes was granular and had eosinophilic staining characteristics. Hepatocellular necrosis was observed in 2/5 100 and 2/5 400 mg/kg/day rats at 4 weeks. By study end, 1/5 rats fed 100 mg/kg/day and 1/5 rats fed 400 mg/kg/day had clear vacuolated foci.

The number of hepatocellular BrdU-labeled nuclei in rats treated with XRD-537nBu is shown in Table 2. Within one week of treatment, the number of labeled nuclei was significantly ($p \leq 0.05$) increased in a dose-related manner in all XRD-537nBu treatment groups that received ≥25 mg/kg/day. The label was located primarily in the

peripheral regions of the lobules. After week 1, the number of labeled nuclei dramatically decreased in all treatment groups and was distributed uniformly throughout the lobule. Although the number of labeled nuclei was statistically significant at weeks 2 and 4 in rats fed 400 mg/kg/day, the increase is not toxicologically significant.

TABLE 2. Number of BrdU-labeled nuclei/1000 hepatocytes in rats fed XRD-537nBu for up to 13 weeks.

Study Week	Dose (mg/kg/day)				
	0	3	25	100	400
1	74.2 ± 31.3	155.4 ± 39.8	334.0* ± 77.9	489.4* ± 84.0	499.0* ± 124.3
2	55.6 ± 9.0	NA	37.2 ± 8.5	64.6 ± 15.1	93.2* ± 23.5
4	19.6 ± 3.2	NA	NA	25.6 ± 2.8	46.8* ± 14.0
13	16.0 ± 2.7	NA	NA	11.8 ± 1.6	28.4 ± 20.2

Data from p. 48 of MRID 45000414
*=p≤0.05
NA=No statistical analysis

III. DISCUSSION

A. DISCUSSION

In this study, none of the animals died and there were no treatment-related clinical signs of toxicity or effects on body weight in male rats fed diets containing 0, 3, 25, 100, or 400 mg/kg/day XRD-537nBu for up to 13 weeks. The test material did, however, induce a dose-related increase in absolute liver weight and liver relative to body weight in rats fed ≥25 mg/kg/day within one week. The increase was sustained and increased slightly through the remainder of the study. The number of BrdU-labeled nuclei revealed by histochemical staining was dramatically increased in a dose-dependent manner after 7 days of treatment in rats fed ≥25 mg/kg/day test material. The increase was not sustained, and by 14 days was not biologically different from control animals. The results are consistent with an initial dramatic increase of DNA synthesis in liver tissue (proliferation) during the first week of treatment followed by hepatocellular hypertrophy at subsequent observations as an explanation for the enlarged livers found in XRD-537nBu-treated rats.

B. STUDY DEFICIENCIES

No deficiencies that would affect the interpretation of the studies were found.