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UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

Office of Prevention, Pesticides
and
Toxic Substances

December 12, 2001

TXR #: 0050330

MEMORANDUM

SUBJECT: Review of Toxicology Studies with Metolachlor/S-Metolachlor Metabolites
Updated Executive Summaries for Metolachlor DERs

PC Code: 108801/108800
DP Barcodes: D260000, D260001, D260002, D260003, D260005, D260393, D260394, D260395, D260396, D260400, D260404, D260407, D260409, D260411, D260414, D260415, D262025, D262026 and D262423

FROM: Virginia A. Dobozy, V.M.D., M.P.H., Veterinary Medical Officer
Reregistration Branch I, Health Effects Division (7509C)
Virginia A Dobozy 12/12/01

THRU: Whang Phang, Ph.D., Branch Senior Scientist
Reregistration Branch I, Health Effects Division (7509C)
12/19/01

TO: Betty Shakleford/Anne Overstreet
Special Review and Reregistration Division (7508C)

Action Requested: Review toxicology studies with Metolachlor/S-Metolachlor Metabolites

Recommendation: Attached are the Data Evaluation Reports (DERs) for the studies. The following table identifies the DP barcode, metabolite and associated MRID number and study type. The Executive Summaries for these studies follow the table. Also included are updated Executive Summaries for MRIDs 00032174, 43244001 (subchronic dog study), 00041283 (developmental rabbit study), 00080897 (reproduction study), 00117597 (mouse carcinogenicity study), 00129377 (rat chronic toxicity/carcinogenicity study), 00151941 (developmental rat study), 40980701, 41164501, 42218601, 42218602 (chronic dog study), 4183301 (21-day dermal study) and 41833102 (dermal penetration study).

DP Barcode	MRID Number(s)	Study Type	Metabolite
D260000	44929504 44929505 44929506 44929507	acute oral acute dermal acute eye irritation acute dermal irritation	CGA 51202
D260001	44929508	dermal sensitization	CGA 51202
D260002	44929509	subchronic toxicity - rat	CGA 51202
D260003	44929510	developmental toxicity - rat	CGA 51202
D260005	44929511 44929512	micronucleus assay gene mutation in bacteria	CGA 51202
D260393	44931704	acute oral	CGA 354743
D260394	44931705	acute dermal	CGA 354743
D260395	44931706	acute eye irritation	CGA 354743
D260396	44931707	acute dermal irritation	CGA 354743
D260400	44931708	dermal sensitization	CGA 354743
D260404	44931709	subchronic toxicity - dog	CGA 354743
D260407	44931710	subchronic toxicity - rat	CGA 354743
D260409	44931711	developmental toxicity - rat	CGA 354743
D260411	44931712 44931713	gene mutation in bacteria micronucleus assay	CGA 354743
D260414	44931714	unscheduled DNA synthesis	CGA 354743
D260415	44931715 44931716 44931717	metabolism metabolism metabolism	CGA 354743 CGA 376944 CGA 376944
D262025	44991101	acute oral toxicity	CGA 354743
D262026	44991102	gene mutation in mammalian cells	CGA 354743
D262423	45001201	gene mutation in mammalian cell	CGA 51202

MRID 44929504

Citation: Hartmann, H. (1991) CGA-51202: Acute oral toxicity in the rat. Ciba-Geigy Limited, Short-term toxicology, 4332 Stein, Switzerland. Laboratory study identification 911337, Novartis No. 412-91, December 12, 1991. MRID 44929504. Unpublished.

EXECUTIVE SUMMARY: In an acute oral toxicity study (MRID 44929504) groups of five male and five female fasted young adult Tif:RAI f (SPF) rats were given a single oral 2000 mg/kg dose of CGA-51202 Technical (Batch No. JD 7069/3, 100%) in 0.5% w/v carboxymethylcellulose in 0.1% w/v aqueous polysorbate 80 and observed for 14 days.

Piloerection, hunched posture, exophthalmos, dyspnea, reduced activity, and/or abnormal respiratory sounds were noted on all animals. The surviving animals recovered by day 8. One male was killed for humane reasons on day 13. No other animals died during the study. Mean body weight changes were normal. The euthanized male had a dilated or inflated stomach, small intestine, and typhlon. The remaining animals had no deviations from normal morphology at necropsy.

The oral LD_{50} for males, females, and combined was > 2000 mg/kg (Toxicity Category III).

This acute oral study is classified as **Acceptable/Guideline** and **satisfies** the guideline requirements for an acute oral study [870.1100] in the rat.

MRID 44929505

Citation: Hartmann, H. (1991) CGA-51202: Acute dermal toxicity in the rat. Ciba-Geigy Limited, Short-term toxicology, 4332 Stein, Switzerland. Laboratory study identification 911338, Novartis No. 413-91, December 12, 1991. MRID 44929505. Unpublished.

EXECUTIVE SUMMARY: In an acute dermal toxicity study (MRID 44929505) approximately 10% of the body surface area of five male and five female young adult Tif:RAI f (SPF) rats was dermally exposed to 1333 mg/kg CGA-51202 Technical (Batch No. JD 7069/3,100%) diluted with distilled water to create a suspension of 33% (w/w) for 24 hours. The animals were observed for 14 days.

None of the animals died during the study. Piloerection and hunched posture were noted in all animals with recovery within 3 days. The mean body weight changes were normal. The study report states that animals had no deviations from normal morphology at necropsy, but no data were submitted.

The dermal LD_{50} for males, females, and combined was > 1333 mg/kg (Toxicity Category II).

This acute dermal study is classified as **Acceptable/Non-guideline** and does not satisfy the guideline

requirements for an acute dermal study [870.1200 (81-2)] in the rat. A limit dose of at least 2000 mg/kg is required by the guideline. However, another study is not required as there are sufficient data for Toxicity Category classification.

MRID 44929506

Citation: Hagemann (1992) CGA-51202: Acute eye irritation/corrosion study in the rabbit. Ciba-Geigy Limited, Short-term toxicology, 4332 Stein, Switzerland. Laboratory study identification 911339, Novartis No. 414-91, March 27, 1992. MRID 44929506. Unpublished.

EXECUTIVE SUMMARY: In a primary eye irritation study (MRID 44929506) 38 mg (0.1 mL weight equivalent) of CGA-51202 Technical (Batch No. JD 7069/3, 100%) was instilled into the conjunctival sac of the left eye of three male rabbits and the eye lids held together for approximately 1 second. The contralateral eye of all rabbits served as control. The eyes were scored for ocular irritation according to the OECD scoring system 1, 24, 48, and 72 hours and days 7, 10, 14, 17, and 21 after test material instillation.

Corneal opacity was found on 3/3 rabbits one hour after test material instillation that persisted through day 10 with resolution on two rabbits by day 14 and on one rabbit by day 21. Iritis was noted on 3/3 rabbits one hour after test material instillation that persisted through 72 hours with resolution on two rabbits by day 7 and on one rabbit by day 10. All rabbits had evidence of conjunctival chemosis at one hour post-instillation, which cleared by either 7, 10 or 14 days. All rabbits had conjunctival redness by one hour post-instillation, which cleared by either 17 or 21 days.

In this study, CGA-51202 Technical was a severe irritant and is in TOXICITY CATEGORY II for primary eye irritation.

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

MRID 44929507

Citation: Hagemann(1991) CGA-51202: Acute dermal irritation/corrosion in the rabbit. Ciba-Geigy Limited, Short-term toxicology, 4332 Stein, Switzerland. Laboratory study identification 911340, Novartis No. 415-91, December 11, 1991. MRID 44929507. Unpublished.

EXECUTIVE SUMMARY: In a primary dermal irritation study (MRID 44929507) three male adult New Zealand white rabbits were dermally exposed to 0.5 g CGA-51202 Technical (Batch No. JD 7069/3, 100%) on a gauze patch moistened with 0.5% w/v carboxymethylcellulose in 0.1% w/v aqueous polysorbate 80 for 4 hours on the flank of the animals. The application sites were scored for erythema and edema 1, 24, 48, and 72 hours after patch removal using the OECD scoring system.

Very slight erythema was noted on 2/3 rabbits one hour following patch removal with resolution by

24-hours.

In this study, CGA-51202 Technical was essentially nonirritating and is in TOXICITY CATEGORY IV for primary dermal irritation.

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

MRID 44929508

Citation: Hagemann (1992) CGA-51202: Skin sensitization test in the guinea pig (Optimization test). Ciba-Geigy Limited, Short-term toxicology, 4332 Stein, Switzerland. Laboratory study identification 911341, Novartis No. 526-92, May 18, 1992. MRID 44929508. Unpublished.

EXECUTIVE SUMMARY: In a dermal sensitization study (MRID 44929508) with CGA-51202 Technical (Batch No. JD 7069/3, 100%), 40 young adult male and female guinea pigs were tested using the Optimization Test.

Following ten intradermal inductions with a 0.1% (w/w) solution of the test material, 5/20 test animals showed positive reactions after intradermal challenge; the control group had no positive reactions after intradermal challenge. Twenty-four hours after epidermal challenge, 3/20 animals showed positive reactions and 11/20 animals showed positive reactions at 48 hours. The sites of the test animals treated with the vehicle did not have any positive reaction. One animal in the control group treated with the test material had a positive reaction 48 hours after epidermal challenge. No animals in the control group treated with the vehicle showed positive reactions. The historical control data demonstrated that 1-chlor-2,4-dinitrophenol positive control and vehicle control animals responded appropriately.

In this study, CGA-51202 Technical was a dermal sensitizer.

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

MRID 44929509

Citation: Schneider, M. (1992) CGA-51202: Final Report. 3-Month oral toxicity study in rats (Administration in food). CIBA-GEIGY Limited, Short/Long-term Toxicology, 4332 Stein, Switzerland. Laboratory Study ID: 911344, July 23, 1992. MRID 44929509. Unpublished.

EXECUTIVE SUMMARY: In a subchronic oral feeding study, (MRID 44929509), CGA-51202 technical (100% a.i.; batch No. JD 7069/3) was fed to groups of 10 male and 10 female albino rats at dose levels of 0, 300, 1000, or 15,000 ppm for 3 months. The average achieved doses for the corresponding groups were 0, 18.7, 62.1, and 1000 mg/kg bodyweight for males, and 0, 20.6, 67.3, and 1020 mg/kg for females.

All animals survived to study termination and no treatment-related clinical signs were observed. There were no treatment-related effects on body weight, food consumption, ophthalmoscopic parameters, or urinalysis. Platelet counts were decreased 16% ($p<0.01$) in high-dose males. Total protein in high-dose males (5% decrease, $p<0.01$) and females (4% decrease, N.S.) was slightly decreased due to decreased globulin in males and decreased albumin and globulin fractions in females. These effects were not considered biologically significant. There were no treatment-related organ weight effects or macroscopic or microscopic lesions. **Under the conditions of this study, the NOAEL is 15,000 ppm in the diet (1000 mg/kg for males, 1020 mg/kg for females, limit dose) based on no biologically significant effects. A LOAEL was not identified.**

This subchronic toxicity study in rats (82-1) is classified as **Acceptable/Guideline.** It satisfies the guideline requirement for a subchronic dietary toxicity study in rodents.

MRID 44929510

Citation: Marty, J.H. (1992) CGA-51202 technical: Rat oral teratogenicity. Ciba-Geigy Limited, Reproduction Toxicology, 4332 Stein, Switzerland. Laboratory Study No. 911351. November 3, 1992. MRID 44929510. Unpublished.

EXECUTIVE SUMMARY: In a developmental toxicity study (MRID 44929510), 24 presumed pregnant Tif: RAI f (SPF) (hybrids of RII/1 × RII/2) rats per group were administered CGA 51202 Technical (100%; Batch No. JD 7069/3) by gavage in 0.5% aqueous sodium carboxy-methylcellulose solution at doses of 0, 10, 100, or 1000 mg/kg/day on gestation days (GD) 6-15, inclusive. Controls were treated with 0.5% sodium carboxymethylcellulose (vehicle). On GD 21, dams were sacrificed, subjected to gross necropsy, and all fetuses examined externally. Approximately one-half of each litter was processed for visceral examination and the remaining one-half was processed for skeletal examination.

One low-dose animal was sacrificed moribund on GD 20 with a urogenital infection. All other animals survived to terminal sacrifice. No clinical signs of toxicity were observed in any animal. Maternal body weights and body weight gains were similar between the treated and control groups throughout the study. Food consumption was not affected by treatment. Maternal necropsy was unremarkable.

Therefore, the maternal toxicity NOAEL is ≥1000 mg/kg/day and the maternal toxicity LOAEL was not identified.

No differences were observed between the treated and control groups for number of corpora lutea, number of implantation sites, live fetuses/dam, pre- and post-implantation losses, fetal body weights, or fetal sex ratios.

No treatment-related external, visceral, or skeletal malformations/variations were observed in any fetus from any group.

The high dose is equivalent to the limit dose for developmental toxicity studies.

Therefore, the developmental toxicity NOAEL is ≥1000 mg/kg/day and the developmental toxicity LOAEL was not identified.

This study is classified as **Acceptable/Guideline** and satisfies the requirements for a developmental toxicity study [870.3700 (§83-3a)] in rats.

MRID 44929511

Citation: Hertner, Th. (1992) CGA-51202: Final Report - Micronucleus test, mouse. CIBA-GEIGY Limited, Genetic Toxicology, Basle, Switzerland. Laboratory study ID 911343, Novartis No. 410-91, August 28, 1992. MRID 44929511. Unpublished.

EXECUTIVE SUMMARY: In a Tif: MAGf (SPF) mouse bone marrow micronucleus assay (MRID 44929511), five mice/sex/dose were treated once via oral gavage with CGA-51202 technical (Batch No. JD 7069/3, 100% a.i.) at doses of 600, 1200 and 2400 mg/kg body weight. In an initial micronucleus assay, bone marrow cells were harvested at 16, 24 and 48 hours post-treatment from test material treated mice and at 24 hours post-treatment from solvent and positive control treated mice. In a second assay, harvest times were 24 and 48 hours post-treatment for the high dose and solvent control mice and 24 hours post-treatment for the intermediate and low dose mice and for the positive control. The vehicle was Arachis oil.

There were signs of toxicity during the study. A preliminary toxicity test was conducted with concentrations up to 3000 mg/kg, the solubility limit using one male and one female per dose. Both animals in each group survived the treatments (three day observation period) but both mice receiving 3000 mg/kg showed respiratory sounds. A confirmatory assay was conducted with 3000 mg/kg using one male and one female. Respiratory sounds were heard in both mice and the male mouse died two days after treatment. An upper dose of 2400 mg/kg was thus selected for the micronucleus assay. In the initial micronucleus assay, data from the 24 and 48 hour harvest times were not acceptable because the analytically determined test material concentrations were too low (the results were not shown). Clinical signs of toxicity seen in mice from the 2400 mg/kg 16 hour sampling time group were respiratory sounds, piloerection and dyspnea. The PCE/NCE ratios did not indicate bone marrow cytotoxicity at any test material dose. No statistically significant increase in the number of micronucleated PCEs over the solvent control value was seen at any CGA-51202 technical concentration at the 16 hour sampling time. The mean percentage of micronucleated PCEs (pooled male and female data in the absence of sex differences) was 0.04%, 0.05% and 0.05% in the 600, 1200 and 2400 mg/kg treatment groups, respectively, compared to the solvent control value of 0.02%. The positive control value was 1.15%, significant at $p<0.05$. A second micronucleus assay was conducted with doses of 600, 1200 and 2400 mg/kg and a 24 hour harvest time. An additional harvest time of 48 hours was used with the 2400 mg/kg group. At the 24 hour harvest time, three females and two males from the 2400 mg/kg groups had respiratory sounds while all other mice in the upper dose groups and all mice in the lower two dose groups appeared normal. In the 48 hour

harvest time groups, all animals except one male had one or more of the following signs of toxicity: respiratory sounds, reduced locomotor activity, piloerection, tremor and dyspnea. The PCE/NCE ratios did not indicate bone marrow cytotoxicity at any test material dose. A statistically significant increase ($p<0.05$) in the mean number of micronucleated PCEs (pooled male and female data) over the solvent control value (0.01%) was seen at 600 (0.08%) and 1200 (0.08%) but not at 2400 mg/kg (0.04%). Although statistically significant due to the very low solvent control value, the actual percentages of micronucleated PCEs were well below the laboratory's criterion for a positive response of 0.20%, thus the results were not considered biologically significant. The positive control value of 0.98% was appropriate. The mean percentage of micronucleated PCEs at the 48 hour harvest time (2400 mg/kg) was 0.05% compared to 0.10% for the solvent control. **There was no biologically significant increase in the frequency of micronucleated polychromatic erythrocytes in bone marrow after any dose or treatment time used in the study.**

This study is classified as **Acceptable/Guideline**. It satisfies the requirement for FIFRA Test Guideline [OPPTS 870.5395 (§84-2)] for *in vivo* cytogenetic mutagenicity data.

MRID 44929512

Citation: Hertner, Th. (1992) *Salmonella* and *Escherichia*/liver-microsome test. CIBA-GEIGY Limited, Genetic Toxicology, Basle, Switzerland. Laboratory study ID 911342, Novartis No. 411-91, March 20, 1992. MRID 44929512. Unpublished.

EXECUTIVE SUMMARY In a reverse gene mutation assay in bacteria (MRID 44929512), strains TA98, TA100, TA1535 and TA1537 of *S. typhimurium* and strain WP2(uvrA) of *E. coli* were exposed to CGA-51202 technical (Batch No. JD 7069/3, 100% a.i.) in DMSO at concentrations of 312.5, 625.0, 1250.0, 2500.0, 5000.0 µg/plate in the presence and absence of mammalian metabolic activation. Two independent assays were conducted and all plating was in triplicate. The S9-fraction was obtained from Aroclor induced male RAI (Tif:RAIf(SPF)) rat liver.

CGA-51202 technical was tested up to a limit concentration of 5000 µg/plate. Cytotoxicity, as based on a reduction in the number of revertants per plate compared to the solvent control value, was seen in a preliminary cytotoxicity assay using TA100 and WP2(uvrA) only at 5000 µg/plate without S9-mix. The number of revertants per plate was 54% of the solvent control value in TA100 and 53% of the solvent control value in WP2(uvrA). A precipitate was seen on the TA100 plate at 5000 µg/plate without S9-mix. In the mutagenicity assays, no cytotoxicity was evident at any test point and no precipitates were seen. CGA-51202 technical did not increase the number of revertants per plate over solvent control values at any tested concentration in any of the five bacterial strains in either mutagenicity assay, with or without S9-mix. The solvent and positive control values were appropriate for the respective strains and within the laboratory's historical control ranges. **There was no evidence of induced mutant colonies over background.**

This study is classified as **Acceptable/Guideline**. It satisfies the requirement for FIFRA Test Guideline 84-2 (OPPTS 870.5100) for *in vitro* mutagenicity [bacterial reverse gene mutation] data.

MRID 44931704

Citation: Cantoreggi, S. (1998) CGA-354743: Acute oral toxicity in the rat (Limit test). Novartis Crop Protection AG, Toxicology, 4332 Stein, Switzerland. Laboratory study identification 981038, Novartis No. 647-98, June 19, 1998. MRID 44931704. Unpublished.

EXECUTIVE SUMMARY: In an acute oral toxicity study (MRID 44931704) groups of five male and five female fasted young adult HanIbm:WIST rats were given a single oral 5000 mg/kg dose of CGA-354743 Technical (metabolite of CGA 24705, Batch No. KI-5408/6, 98.0% a.i.) in distilled water and observed for 14 days.

No rats died and all rats had normal body weight gains during the study. No remarkable clinical observations were noted during the study and no remarkable observations were noted at necropsy.

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

CGA-354743 Technical is in TOXICITY CATEGORY IV based on the LD_{50}.

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

MRID 44931705

Citation: Cantoreggi, S. (1998) CGA-354743: Acute dermal toxicity in the rat (Limit test). Novartis Crop Protection AG, Toxicology, 4332 Stein, Switzerland. Laboratory study identification 981039, Novartis No. 645-98, June 19, 1998. MRID 44931705. Unpublished.

EXECUTIVE SUMMARY: In an acute dermal toxicity study (MRID 44931705) approximately 10% of the body surface area of five male and five female young adult rats was dermally exposed to 2000 mg/kg (Limit Test) CGA-354743 Technical (metabolite of CGA 24705, Batch No. KI-5408/6, 98.0% a.i.) moistened with distilled water for 24 hours. The animals were observed for 14 days.

None of the animals died during the study. No remarkable clinical observations or local irritation were noted on any rats. With the exception of one female that lost weight during the first week, all animals had normal body weight gains. No observable abnormalities were noted at necropsy.

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

CGA-354743 Technical is in TOXICITY CATEGORY III based on the LD_{50}.

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

MRID 44931706

Citation: Cantoreggi, S. (1998) CGA-354743: Acute eye irritation/corrosion in the rabbit. Novartis Crop Protection AG, Toxicology, 4332 Stein, Switzerland. Laboratory study identification 981041, Novartis No. 646-98, June 19, 1998. MRID 44931706. Unpublished.

EXECUTIVE SUMMARY: In a primary eye irritation study (MRID 44931706) 41 mg (0.1 mL weight equivalent) of CGA-354743 Technical (metabolite of CGA 24705, Batch No. KI-5408/6, 98.0% a.i.) was instilled into the conjunctival sac of the left eye of three male and three female rabbits and the eye lids held together for approximately 1 second. The eyes were washed with distilled water for one minute after the 24 hour reading. The contralateral eye of all rabbits served as control. The eyes were scored for ocular irritation according to the OECD/EEC/MAFF scoring system 1, 24, 48, and 72 hours after instillation.

Corneal opacity (grade 1-2) was found in 5/6 rabbits one hour after test material instillation that persisted through 24 hours in 4/6 animals. By 48 hours, 4/6 rabbits had corneal opacity with resolution in 2/6 rabbits by 72 hours, in 1/6 rabbits by day 7 and in 1/6 rabbits by day 10. Iritis (grade 1) was noted on 3/6, 4/6, 2/6, and 2/6 rabbits 1, 24, 48, and 72 hours, respectively, after test material instillation; resolution occurred by 7 days. The test material induced conjunctival redness (grades 1 or 2) in all rabbits within one hour of treatment with resolution in 5/6 rabbits within 7 days and in 1/6 rabbits within 10 days. Conjunctival chemosis (grade 1 or 2) was noted in 6/6 rabbits one hour after treatment which resolved by day 7.

In this study, CGA-354743 Technical was a moderate irritant and is in TOXICITY CATEGORY II for primary eye irritation.

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

MRID 44931707

Citation: Cantoreggi, S. (1998) CGA-354743: Acute dermal irritation/corrosion in the rabbit. Novartis Crop Protection AG, Toxicology, 4332 Stein, Switzerland. Laboratory study identification 981040, Novartis No. 644-98, May 1, 1998. MRID 44931707. Unpublished.

EXECUTIVE SUMMARY: In a primary dermal irritation study (MRID 44931707) three male and three female adult New Zealand white rabbits were dermally exposed to 0.5 g CGA-354743 Technical (metabolite of CGA 24705, Batch No. KI-5408/6, 98.0% a.i.) on a gauze patch moistened with distilled water for 4 hours on the flank of the animals. The animals were scored 1, 24, 48, and 72 hours after patch removal. Irritation was scored by the method of Draize.

Very slight erythema was noted on 4/6 rabbits (1 male and 3 females) one hour following patch removal with resolution on 2/6 rabbits by 24 hours, on 1/6 rabbits by 48 hours, and on 1/6 rabbits

by 72 hours. There was no edema observed. The primary dermal irritation index was 0.29. The female rabbit which had the longest duration of erythema lost weight during the 72-hour period.

In this study, CGA-354743 Technical was essentially nonirritating and is in TOXICITY CATEGORY IV for primary dermal irritation.

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

MRID 44931708

Citation: Cantoreggi, S. (1999) CGA-354743: Skin sensitization test in the guinea pig (Buehler test). Novartis Crop Protection AG, Toxicology, 4332 Stein, Switzerland. Laboratory study identification 981042, Novartis No. 1097-99, February 3, 1999. MRID 44931708. Unpublished.

EXECUTIVE SUMMARY: In a dermal sensitization study (MRID 44931708) with CGA-354743 Technical (metabolite of CGA 24705, Batch No. KI-5408/6, 98.0% a.i.), 10 young adult male and 10 female guinea pigs were tested using the Buehler Test. An additional five animals/sex served as a vehicle control group and five/sex as a naive control group.

After the first induction, irritation (slight or slight to moderate confluent erythema) at the application site was observed in 13/20 and 18/20 animals at the 24-hour and 48-hour evaluations, respectively. After the second induction, irritation was observed in 17/20 and 18/20 animals at the 24-hour and 48-hour examinations, respectively. After the third induction, all animals had evidence of irritation. The vehicle control animals had no reaction. One female test animal had slight confluent erythema at 48 hours and another female test animal had slight confluent erythema 24 hours after challenge that persisted through 48 hours. No rechallenge was conducted. The flanks of the test animals treated with vehicle had no reaction. The vehicle control and naive control animals had no reaction after challenge. The study report included a positive control study carried out within six months of the current study. The 2-mercaptobenzothiazole positive control and vehicle control animals responded appropriately.

In this study, CGA-354743 Technical was a weak dermal sensitizer.

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

MRID 44931709

Citation: Altmann, B. (1999) 3-Month subchronic, comparative oral toxicity study in beagle dogs. Novartis Crop Protection AG, Toxicology, Stein, Switzerland. Laboratory Study Identification 971089, January 25, 1999. MRID 44931709. Unpublished.

EXECUTIVE SUMMARY: In a 90-day subchronic oral toxicity study (MRID 44931709), CGA-354743 technical (Batch Nos. KI-5408/4 and KI-5408/5, 99% a.i.) was administered to 4 purebred beagle dogs/sex/dose by capsule at dose levels of 0, 50, 200, 500, and 1000 mg/kg/day for 13 weeks. An additional group of 4 males and 4 females received parent compound (CGA-77102 technical, Batch No. P.501001, 98.5% a.i.) at 200 mg/kg/day for 13 weeks.

There were no significant treatment related effects on mortality, body weight, food consumption, food conversion ratios, ophthalmological findings, hematology and urinalysis parameters, or gross and histopathological findings. Vomiting did occur at a higher incidence in females treated with 1000 mg/kg/day of CGA-354743. Clinical signs in animals treated with CGA-77102 included vomiting, salivation and hematuria. Mean alkaline phosphatase activity was slightly increased in males receiving 1000 mg/kg/day CGA-354743 at weeks 7 and 13 to levels which were less than double the pretest mean for this group. This finding correlated with slightly increased absolute liver weights, but there were no corresponding histopathological findings, or toxicologically significant increases in other biochemistry parameters. In females, mean ALP activities remained within the reference range for untreated animals and mean GGT activity exceeded the reference range only at week 13 and only for the 500 mg/kg/day CGA-354743 group. Absolute liver weights and liver weights relative to body weights were increased in females receiving 500 and 1000 mg/kg/day. In the absence of corresponding histopathological findings or biologically significant increases in biochemistry parameters consistent with adverse hepatic effects, this finding is not considered toxicologically significant.

Mean ALP and GGT activities were significantly increased in both sexes at weeks 7 and 13 given CGA-77102. In addition, ALT activity of males was increased at weeks 7 and 13. Absolute and relative liver weights were significantly increased in males and females. There were small increases in the incidences and severity of bile duct hyperplasia, perilobular fatty change in the livers of both sexes, and cystic hyperplasia of the gallbladder occurred only in the parent compound group.

The results appear to indicate that CGA-354743 may have effects (vomiting, slight increases in ALT and liver weight) similar to those of its parent compound, CGA-77102; however, at the limit dose, 1000 mg/kg/day, the effects observed were so slight and of questionable toxicological significance in CGA-35743-treated dogs that a definitive comparison of the two compounds cannot be made.

Based on the data presented in this study, the LOAEL was not determined, and the NOAEL was greater than or equal to 1000 mg/kg/day.

This subchronic oral toxicity study in dogs is classified as **Acceptable/Guideline** and satisfies the guideline requirements for a subchronic oral study [OPPTS: 870.3150 (§82-1b)] in dogs since the limit dose was tested.

MRID44931710

Citation: Bachmann, M. (1999) CGA-354743: Final report. 3-month oral toxicity study in rats (Administration in food). Novartis Crop Protection AG, Toxicology, 4332 Stein, Switzerland. Study # 971142, Novartis # 1187-98. January 26, 1999. MRID 44931710. Unpublished.

EXECUTIVE SUMMARY: In a 90-day subchronic oral toxicity limit study (MRID 44931710), groups of 10 male and 10 female Crl: CD BR rats were given CGA-354743 (Lot/Batch # KI-5408/6, 98% a.i.) administered in the diet at concentrations of 0, 360, 1200, 6000, or 20,000 ppm. These concentrations were equivalent to 0, 25.1, 86.2, 427.0 or 1545.0 mg/kg/day for males and 0, 28.4, 98.3, 519.0 and 1685.0 mg/kg/day for females. An additional 10 male and 10 female rats were given CGA-77102 (s-Metolachlor)(Lot/Batch# P.501001, 98.5% a.i.) administered in the diet at 5000 ppm (equivalent to 429 mg/kg/day for males and 563 mg/kg/day for females). The study was designed to assess the subchronic oral toxicity of CGA-354743 technical and to compare its toxic effects with those of its parent compound, CGA-77102 technical.

No deaths or clinical signs of toxicity occurred during this study. In addition, no statistically significant changes in body weight, body weight gain, food consumption, food efficiency, ophthalmologic examination, urinalysis, or histopathology was reported for animals fed CGA-354743. Limited and sporadic statistically significant changes in hematology, clinical chemistry, water intake and organ weight data were not dose-dependent, and were of questionable toxicological and biological importance.

Dietary exposure to CGA-77102 produced a statistically significant decreased body weight gain (-20%, $p \leq 0.01$) in males during week 1 only. Females exposed to CGA-77102 showed decreased body weight gain (-19%) by week 13, but these changes were not statistically significant. The food efficiency of rats fed CGA-77102 was decreased relative to their respective control animals. Male and female rats had increased absolute and relative liver weights. These results are consistent with a mild liver hypertrophy in females.

Based on the data presented in this study, the NOAEL is ≥20,000 ppm (1543 mg/kg/day and 1685 mg/kg/day for females) for CGA-354743. A LOAEL could not be established.
At 5000 ppm (429 mg/kg/day in males and 563 mg/kg/day in females) CGA-77102 , there evidence of decreased body weight gain and food efficiency, increased absolute and relative liver weights and an increased incidence of hepatic centrilobular hypertrophy, although the effects were mild.

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

MRID 44931711

Citation: Doubovetzky, M. (1999) CGA-354743 technical: Rat oral teratogenicity. Novartis Crop

Protection AG, Toxicology, 4332 Stein, Switzerland. Laboratory Study No. 981009. January 25, 1999. MRID 44931711. Unpublished.

EXECUTIVE SUMMARY: In a developmental toxicity study (MRID 44931711), 28 presumed pregnant Wistar B: Hanlbm:WIST rats per group were administered CGA 354743 Technical (98%; Batch No. KI-5408/6) by gavage in 0.5% aqueous sodium carboxymethylcellulose in 0.1% aqueous polysorbate 80 at doses of 0, 250, 500, or 1000 mg/kg/day on gestation days (GD) 6-15, inclusive. Controls were treated with 0.5% sodium carboxymethylcellulose in 0.1% aqueous polysorbate 80 (vehicle). On GD 21, dams were sacrificed, subjected to gross necropsy, and all fetuses examined externally. Approximately one-half of each litter was processed for visceral examination and the remaining one-half was processed for skeletal examination.

All animals survived to terminal sacrifice. No clinical signs of toxicity were observed in any animal. Maternal body weights, body weight gains, and food consumption were similar between the treated and control groups throughout the study. Maternal necropsy was unremarkable.

Therefore, the maternal toxicity NOAEL is ≥1000 mg/kg/day and the maternal toxicity LOAEL was not identified.

No differences were observed between the treated and control groups for number of corpora lutea, number of implantation sites, live fetuses/dam, pre- and post-implantation losses, fetal body weights, or fetal sex ratios.

No treatment-related external, visceral, or skeletal malformations/variations were observed in any fetus from any group.

The high dose is equivalent to the limit dose for developmental toxicity studies.

Therefore, the developmental toxicity NOAEL is ≥1000 mg/kg/day and the developmental toxicity LOAEL was not identified.

This study is classified as **Acceptable/Guideline** and satisfies the requirements for a developmental toxicity study [870.3700 (§83-3a)] in rats.

MRID 44931712

Citation: Ogorek, B. (1996) CGA-354743: Final report - *Salmonella* and *Escherichia*/mammalian-microsome mutagenicity test. CIBA-Geigy Limited, Genetic Toxicology, Basle, Switzerland. Laboratory study ID 951133, Novartis No. 813-95, January 15, 1996. MRID 44931712. Unpublished.

EXECUTIVE SUMMARY: In a reverse gene mutation assay in bacteria (MRID 44931712), strains TA98, TA100, TA102, TA1535 and TA1537 of *S. typhimurium* and strain WP2(uvrA) of *E. coli*

were exposed to CGA-354743 tech. (Batch No. RV-2816/1, 95% a.i.) in DMSO at concentrations of 312.5, 625.0, 1250.0, 2500.0 and 5000.0 µg/plate in the presence and absence of mammalian metabolic activation (S9-mix). The S9-fraction was obtained from Aroclor 1254 induced male RAI (Tif:RAIf (SPF)) rat liver.

CGA-354743 tech. was tested up to a limit concentration of 5000 µg/plate. No cytotoxicity, as measured by thinning or absence of the background lawn of bacteria or by a reduction in the number of revertants per plate compared to the solvent control values, was seen in the preliminary cytotoxicity test or in the mutagenicity tests at concentrations up to 5000 µg/plate, with or without S9-mix. An initial and a confirmatory mutagenicity assay was conducted and all plating was in triplicate. The number of revertants per plate was not increased over solvent control values in any strain at any tested concentration, with or without S9-mix, in either assay. The positive and solvent control values were appropriate in the respective strains and within the laboratory's historical control ranges. **There was no evidence of induced mutant colonies over background.**

This study is classified as **Acceptable/Guideline**. It satisfies the requirement for FIFRA Test Guideline 84-2 (OPPTS 870.5100) for *in vitro* mutagenicity [bacterial reverse gene mutation] data.

MRID 44931713

Citation: Deparade, E. (1998) CGA-354743: Final Report - Micronucleus test, mouse. Novartis Crop Protection AG, Toxicology, Genetic Toxicology, Basle, Switzerland. Laboratory Study ID 981016, Novartis No. 1190-98, October 19, 1998. MRID 44931713. Unpublished.

EXECUTIVE SUMMARY: In a ICO:CD1 (CRL) mouse bone marrow micronucleus assay (MRID 44931713), five mice/sex/dose were treated once each via oral gavage with CGA-354743 tech. (Batch No. KI5408/6, 98 ± 2% a.i.) at doses of 1250, 2500 and 5000 mg/kg body weight. Bone marrow cells were harvested at 16, 24 and 48 post-treatment from the high dose and negative control groups and at 24 hours only from the intermediate and low dose and positive control groups. The vehicle was bidistilled water.

There were no signs of toxicity in the preliminary toxicity assay (5000 mg/kg only) or at any dose or sampling time in the micronucleus assay. The upper dose was the limit dose for this assay and also the solubility limit. No bone marrow cytotoxicity, based on the PCE/NCE ratio was evident. There was no statistically significant increase in the percentage of micronucleated PCEs in any CGA-354743 tech. treated group when compared to the solvent control groups and there were no sex-related differences seen. The mean percentage of micronucleated PCEs (pooled male/female data) was 0.04% in all treatment groups except the 5000 mg/kg, 24 hour harvest time group in which the percentage was 0.07%. The solvent control values were 0.03, 0.03 and 0.01% at 16, 24 and 48 hours, respectively while the positive control value was 1.07% (24 hour harvest time only). The positive and solvent controls induced the appropriate responses. **There was no significant increase in the frequency of micronucleated polychromatic erythrocytes in bone marrow after any dose or treatment time.**

This study is classified as **Acceptable/Guideline**. It satisfies the requirement for FIFRA Test Guideline [OPPTS 870.5395 (§84-2)] for *in vivo* cytogenetic mutagenicity data.

MRID 44931714

Citation: Ogorek, B. (1998) CGA-354743: Final Report - Autoradiographic DNA repair test on rat hepatocytes (OECD Conform) *in vitro*. Novartis Crop Protection AG, Toxicology, Genetic Toxicology, Basle, Switzerland. Laboratory Study ID 981017, Novartis No. 1189-98, November 23, 1998. MRID 44931714. Unpublished.

EXECUTIVE SUMMARY: In an unscheduled DNA synthesis (UDS) assay (MRID 44931714), primary rat hepatocyte cultures were exposed to CGA-354743 tech. (Batch No. KI-5408/6, 98% a.i.) in bidistilled water at concentrations of 9.77, 39.06, 156.25, 625.00, 2500.00, 5000.00 µg/mL for 16 to 18 hours in an initial assay and to concentrations of 78.13, 156.25, 312.50, 625.00, 1250.00, 2500 µg/mL for 16 to 18 hours in a confirmatory assay. Primary hepatocytes were obtained from healthy male HANIbm:WIST(SPF) rats.

CGA-354743 tech. was tested up to cytotoxic concentrations based on cell morphology changes and reduced cell viability. A cytotoxicity test at concentrations ranging from 4.88 to 5000.00 µg/mL showed a general trend of decreasing cell viability with increasing test material concentration, decreasing from 81% viable cells at 4.88 µg/mL to 57% at 5000.00 µg/mL. Viability of the solvent controls was 88%. Effects on cell morphology were seen at concentrations of 39.06 and higher, although, sufficient cells with good morphology were available for UDS determination at all test material concentrations. One hundred and fifty cells (50/slide) were scored for UDS per treatment group. There was no increase in the net nuclear grain count, the net grain count of cells in repair or in the percentage of cells in repair over solvent control values at any concentration in either assay. The mean net nuclear grain counts remained below 1.0 at all concentrations in both assays. The mean net nuclear grain counts of the solvent controls were 0.5 and -0.4 in the initial and confirmatory assays, respectively, while those of the positive controls were 12.3 and 9.3 in the initial and confirmatory assays, respectively. The positive and solvent controls induced the appropriate response. **There was no evidence that unscheduled DNA synthesis, as determined by radioactive tracer procedures (nuclear silver grain counts), was induced.**

This study is classified as **Acceptable/Guideline**. It satisfies the requirement for FIFRA Test Guideline (OPPTS 870.5550; 84-2) for other genotoxic mutagenicity data.

MRID 44931715

Citation: Mewes, K. (1998). Determination of the soil metabolites CGA-354743, CGA-368208, and CGA-357704 in excreta of rats administered [Phenyl-U-^{14}C]CGA-77102. Novartis Crop. Protection, AG, CH-4002 Basle, Switzerland. Laboratory Study Identification 030AM07, Novartis Number 796-97. May 6, 1998. MRID 44931715. Unpublished.

EXECUTIVE SUMMARY:In a metabolism study (MRID 44931715), groups of three male and three female rats were each given [phenyl-U-^{14}C] CGA 77102 (batch no. ILS-143.1; purity 98.9%) and non-radiolabeled CGA 77102 (batch no. AMS 757-101; purity 99.8%) to provide a total single oral dose of 0.5 or 100 mg/kg. Urinary and fecal excretion were monitored over 72 hours and major metabolites identified and quantified. The study focused on evaluating the presence of the metabolites CGA 354743, CGA 368208, and CGA 357704 in the excreta of rats.

There were no deaths or overt signs of toxicity attributed to the test material. Actual administered doses were 3-8% greater than nominal. Overall recovery of administered radioactivity was an acceptable 93.83-99.18%. Urinary excretion and carcass burden data implied that absorption was approximately 38-49% of the administered dose. Most (86.5-91.7%) of the radioactivity recovered at 72 hours post was associated with the urine and feces. The available data suggested that urinary excretion was slightly greater in female rats than male rats (42% for low- and high-dose females as compared to 30% and 32% of low- and high-dose males, respectively), and fecal elimination was expectedly less (approximately 13-15%) for females than for males. However, the small sample size (three rats per sex) precludes a definitive assessment of gender-specific differences in excretory pattern. Time-course data for absorption and excretion were not provided. Based upon the residual radioactivity in the carcasses, accumulation in the tissues was less than 10% of the administered dose at 72 hours after administration.

Both urinary and fecal metabolites were identified and quantified. For both routes of elimination, three major metabolites were identified but none represented more than 0.25% of the administered dose. Characterization of these metabolites and comparison to known reference standards revealed them to be CGA 357704, CGA 354743, and CGA 368208. In the feces, CGA 357704 and CGA 354743 represented a notably greater percent of the administered dose than in the urine. The amounts of CGA 368208 were similar in the feces and urine. Biliary excretion experiments were not performed and, therefore, no assessment can be made regarding biliary or gut microflora as the source of these biotransformation products in the feces.

This metabolism study in rats is **Acceptable/Non-guideline**. Although not satisfying the requirements for a Metabolism and Pharmacokinetics study [OPPTS 870.7485 (85-1)], the study was well designed and conducted, and provided supplemental data regarding the quantitation and identification of urinary and fecal metabolites in rats given a single oral dose of CGA 77102.

MRIDs 44931716 and 44931717

Citations: Muller, T. (1997). Disposition of [Phenyl-U-^{14}C]-CGA-376944, a sulfonic acid soil metabolite of CGA-77102, in the rat. Novartis Crop Protection AG, CH-4002 Basle, Switzerland. Laboratory Study No. 030AM06, Novartis No. 795-97, November 25, 1997. MRID 44931717. Unpublished.

Hassler, S. (1999). Disposition of [Phenyl-U-^{14}C]-CGA-376944, a sulfonic acid soil metabolite of CGA-77102, in bile-duct cannulated rats after oral administration. Novartis Crop Protection AG,

CH-4002 Basle, Switzerland. Laboratory Study No. 030AM08, Novartis No. 1066-99. MRID 44931716. Unpublished.

EXECUTIVE SUMMARY: In a metabolism study (MRIDs 44931716 and 44931717), groups of four male and female and six male Tif: RAI f (SPF) rats were given single oral doses of [Phenyl-U-^{14}C]-CGA-376944 (0.5 mg/kg nominal; Batch No. ILS-125.4 radiochemical purity >95.5%), for the metabolism and bile-duct cannulation studies, respectively.

There were no deaths or overt signs of toxicity that could be attributed to the test material. Weight loss in bile-duct cannulated rats was attributed to surgical trauma. Radioactivity inventory indicated an acceptable 96.46-99.01% recovery of the administered dose among the experimental groups. Based on urinary excretion, biliary excretion, and carcass burden, 17.35% of the administered radioactivity was absorbed following a single oral dose of 0.5 mg/kg of [Phenyl-U-^{14}C]-CGA-376944. Absorption was rapid but limited and most of the absorbed radioactivity (92.3%) was excreted within 24 hours; primarily in the bile. At 72 hours, measurable radioactivity was found only in the liver of non-cannulated rats. Carcass burdens accounted for <0.01% of the administered dose at necropsy.

Fecal elimination was the major route of excretion, accounting for most of the administered oral dose in non-cannulated rats (94.24-96.27%). Fecal excretion was rapid with 98.8-99.2% of the fecal excretion occurring within 24 hours post-dosing. Urinary excretion, accounted for only 2.1-4.4% of the dose in non-cannulated rats and 5.3% in bile-duct cannulated rats. Urinary excretion was rapid and nearly complete within 24 hours of dosing. Biliary excretion represented 11.5% of the administered dose at 48 hours. The majority of biliary excretion (99.2%) occurred within 24 hours after dosing. In bile-duct cannulated animals, an additional 76.8% of the administered dose was excreted in the feces. Based on these data, biliary excretion is a contributor to fecal elimination of the test material. This appears consistent with the occurrence of enterohepatic circulation via the hepatic portal system and bile-duct. Only a minor percentage of the dose (5.3%) appeared to enter the systemic circulation where it was rapidly excreted by the kidneys. No biologically relevant gender-related differences were detected in the oral dose groups.

Blood pharmacokinetic parameters could not be calculated due to low blood concentrations and rapid clearance of the administered dose. Blood levels of radioactivity peaked in both sexes within one hour post-dosing.

It is evident from the results of this study that the test material undergoes limited but rapid absorption and nearly complete excretion within 24 hours. The primary route of excretion is via the feces with biliary excretion products representing about 15.0% of the fecal excretion products. At the dose tested, [^{14}C]-CGA-376944 exhibits little potential for accumulation in the tissues. There were no significant gender-related differences in absorption and disposition of the test material. The unchanged test material accounted for >90% of the dose in both males and females. The unknown metabolites of urine and feces, separated by TLC accounted for 0.2-2.8% of the administered radioactivity and were not characterized further.

This combined metabolism study in rats is **Acceptable/Guideline** and satisfies the requirements for a Metabolism and Pharmacokinetics study [OPPTS 870.7485 (§85-1)].

MRID 44991101

Citation: Winkler, G. (1995) Acute oral toxicity in the rat (limit test). Short-term Toxicology, Novartis Crop Protection, Inc. (Formerly Ciba-Geigy Limited), 4332 Stein, Switzerland. Laboratory study identification 816-95, December 5, 1995. MRID 44991101. Unpublished.

EXECUTIVE SUMMARY: In an acute oral toxicity study (MRID 44991101) five male and five female fasted young adult Tif:RAI f (SPF) rats were given a single oral 2000 mg/kg dose of CGA-354743 Tech. (95%, a.i., Batch No. RV-2816/1) in 0.5% w/v Carboxymethylcellulose in 0.1% w/v aqueous polysorbate 80 and observed for 14 days.

No animals died during the study. Piloerection and hunched posture were noted from all rats one hour post dosing with recovery by day 3. With the exception of one female that lost weight during the second week, all rats had normal body weight gains. No observable abnormalities were noted at necropsy.

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

CGA-354743 Tech. is in TOXICITY CATEGORY III based on the LD_{50}.

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

MRID 44991102

Citation: Ogorek, B. (1999) CGA-354743 tech. (Metabolite of CGA-24705): Final report - Gene mutation test with Chinese hamster cells V79. Genetic Toxicology, Novartis Crop Protection AG, CH-4002 Basel, Switzerland. Laboratory Study ID 981018, Novartis No. 1193-98, January 19, 1999. MRID 44991102. Unpublished.

EXECUTIVE SUMMARY: In a mammalian cell gene mutation assay at the HPRT locus (MRID 44991102), Chinese hamster V79 cells in culture were exposed to CGA-354743 tech. in bidistilled water at concentrations of 185.19, 555.56, 1666.67, 5000.00 µg/mL in the presence and absence of mammalian metabolic activation (S9-mix). The S9-fraction was obtained from Aroclor 1254 induced male Tif:RAI/SPF rat liver.

CGA-354743 tech. was tested up to a weakly cytotoxic limit concentration of 5000 µg/mL. In a preliminary cytotoxicity test, the number of viable cells was reduced approximately 27% and 32%, compared to the solvent control group, at 5000.00 µg/mL with and without S9-mix, respectively. An initial and a confirmatory assay were conducted using two cultures per dose, four dishes per

culture. In the presence of S9-mix, small but statistically significant increases in mean mutant frequencies over the solvent control value were seen in the initial mutation assay at concentrations of 555.56 μg/mL (4.10 per 10^6 viable cells, $0.002<p<0.01$) and 5000.00 μg/mL (5.35 per 10^6 viable cells, $p<0.001$) but not at 1666.67 μg/mL (3.17 per 10^6 viable cells). The mutant frequency of the solvent control was 2.80×10^6 viable cells. Results in the confirmatory assay with S9-mix were similar with small but statistically significant increases in mean mutant frequencies over the solvent control value of 1.58 per 10^6 viable cells seen at 555.56 μg/mL (2.60 per 10^6 viable cells, $0.02<p<0.05$), 1666.67 (3.40 per 10^6 viable cells, $0.002<p<0.01$) and 5000.00 μg/mL (2.91 per 10^6 viable cells, $0.002<p<0.01$). The mean mutant frequency of the DMN positive control was 118.27 per 10^6 viable cells in the initial assay and 116.68 per 10^6 viable cells in the confirmatory assays. In the absence of S9-mix, statistically significant increases in mutant frequencies were seen at 555.56 and 5000.00 μg/mL CGA-35473 but not at 1666.67 μg/mL in both the initial and the first confirmatory assays. In the initial assay, the mean mutant frequency at 5000.00 μg/mL was 19.7 per 10^6 viable cells ($p<0.001$) compared to the solvent control value of 3.66 per 10^6 viable cells and the normalized mean number of mutants per flask was 39.41 compared to the solvent control value of 7.33. The results at 5000.00 μg/mL met the laboratory's criteria for a positive response. In the first confirmatory assay, the statistically significant increase in the mutant frequency over the solvent control value seen at 5000.00 μg/mL ($0.001<p<0.002$) was not accompanied by a normalized mean number of mutants per flask of at least 20 more than the solvent control (18.63 vs 6.63 for the solvent control), thus not meeting the criteria for a positive response. A second confirmatory assay was performed without S9-mix and small but statistically significant increases in mutant frequency were seen at 555.56 and 1666.67 μg/mL but not at 5000.00 μg/mL; therefore, the positive result seen in the initial assay could not be confirmed. There was a statistically significant relationship between dose and mutant frequency in the initial and first confirmatory assay ($p<0.001$) but not in the second confirmatory assay. Although the increases in mutant frequencies over solvent control values seen in this study were statistically significant, all mutant frequencies in test material treated cultures were well within the laboratory's historical solvent control range of 1.01 to 15.68 per 10^6 viable cells and below the historical solvent control mean mutant frequency of 5.46 per 10^6 viable cells (with the one exception at 5000.00 μg/mL without S9-mix). In addition, the solvent control values seen in both the initial and confirmatory assays were near the bottom of the historical control range. The normalized mean number of mutants per flask did not meet the laboratory criterion of 20 or more greater than the solvent control value at any dose with S9-mix or at any dose without S9-mix except at 5000.00 μg/mL as described. The statistically significant differences seen are thus unlikely to be biologically significant. It is of note that none of the assay results satisfied the generally accepted criteria for a positive response in this test system (i.e., reproducibility, dose response and/or minimum of 3-fold increase over background).[1] The positive and solvent controls induced the appropriate response. **There was suggestive (statistical) evidence of a possible induction of mutant colonies over background; however, the results are unlikely to be biologically**

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[1]Nestman, ER, Brillinger RL, Gilman JPW, Rudd CJ, Swierenga SHH (1991). Recommended protocols based on a survey of current practice in genotoxicity testing laboratories: II Mutation in Chinese hamster ovary, V79 Chinese hamster lung and L5178Y mouse lymphoma cells. Mutat Res 246:255-284.

significant because the absolute numbers of mutant colonies were low and within the testing laboratory's historical solvent control ranges.

This study is classified as **Acceptable/Guideline**. It satisfies the requirement for FIFRA Test Guideline OPPTS 870.5300 [§84-2] OPPTS 870.5300 for in vitro mutagenicity (mammalian forward gene mutation) data.

MRID 45001201

Citation: Ogorek, B. (1999) CGA-51202 tech. (metabolite of CGA-24705): Final report; Gene mutation test with Chinese hamster cells V79. Genetic Toxicology, Novartis Crop Protection AG, CH-4002 Basel, Switzerland. Laboratory Study ID: 981112, Novartis No. 1192-98, January 18, 1999. MRID 45001201. Unpublished.

EXECUTIVE SUMMARY: In a mammalian cell gene mutation assay at the HPRT locus (MRID 45001201), Chinese hamster V79 cells cultured *in vitro* were exposed to CGA 51202 tech. (Batch No. JD 7069/3, 100% a.i.) in DMSO at concentrations of 500, 1000, 2000 and 4000 µg/mL in the presence and absence of mammalian metabolic activation (S9-mix). A confirmatory assay was conducted at test material concentrations of 375, 750, 1500 and 3000 µg/mL. The S9-fraction was obtained from Aroclor 1254 induced male Tiff:RAI/SPF rat liver.

CGA 51202 tech. was tested up to cytotoxic concentrations. The upper concentrations in both the initial and confirmatory assays, with and without S9-mix, killed virtually all the cells. Statistically significant increases in mean mutant frequency were seen in the initial assay with S9-mix at 500 µg/mL (6.66×10^{-6}) and 1000 µg/mL (5.56×10^{-6}) compared to the solvent control value of 4.02×10^{-6} and without S9-mix at 500 µg/mL (15.35×10^{-6}) compared to the solvent control value of 12.90×10^{-6}. The increases were small and the actual mean mutant frequencies were within the range of historical solvent control values. No positive dose-response was seen and no statistically significant increases in mean mutant frequencies were seen in the confirmatory assay. The solvent and positive controls induced the appropriate response. **There was no evidence of a biologically significant induction of mutant colonies over background.**

This study is classified as **Acceptable/Guideline**. It satisfies the requirement for FIFRA Test Guideline [OPPTS 870.5300 (§84-2)] for *in vitro* mutagenicity (mammalian forward gene mutation) data.

Updated Executive Summaries for Metolachlor DERs

MRIDs 00032174 and 43244001

Estes, F.L. (1980) 6-Month Chronic Oral Toxicity Study in Beagle Dogs. International Research and Development Corporation (IRDC), Mattawan, MI., Study Number 382-054, May 21, 1980. MRID No. 00032174. Unpublished.

EXECUTIVE SUMMARY:

In a subchronic oral toxicity study (MRIDs 00032174 and 43244001), metolachlor (96.8% ai) was administered in the diet to Beagle dogs (8/sex/group for control and high dose groups; 6/sex/group for low- and mid-dose groups) at dose levels of 0, 100, 300 or 1000 ppm (males: 0, 2.92, 9.71 and 29.61 mg/kg/day, respectively; females: 0, 2.97, 8.77 and 29.42 mg/kg/day, respectively) for six months.

There were no deaths or clinical signs of toxicity. Mean body weight gain was decreased during weeks 0-13 and 0-26 in the 1000 ppm group males (55-63% decrease) and females (44-50% decrease), although the changes were not statistically significant. Mean overall food consumption was not affected in the 1000 ppm group males but was slightly decreased (9%) in the 1000 ppm females. There was a significant decrease in the activated partial thromboplastin time (APTT) in the 300 and 1000 ppm group males and 300 ppm group females but the findings were not considered toxicologically significant because the decrease was slight and not dose-related. Alkaline phosphatase was signifcantly increased in the 300 ppm and 1000 ppm group males and females at week 26; however, the effect was not considered toxicologically significant due to the small magnitude of the increase and the lack of accompanying necropsy findings.

The LOAEL was 1000 ppm (males/females: 29.61/29.42 mg/kg/day) based on decreased body weight gain. The NOAEL was 300 ppm (males/females: 9.71/8.77 mg/kg/day).

The study is classified as **Acceptable/guideline** and **satisfies** the guideline requirements for a subchronic toxicity study in dogs (82-1; OPPTS 870.3150). The study was conducted for six months, whereas the guidelines require 90 days of dosing. However, toxicity parameters, with the exception of necropsy, were also evaluated at 90 days in the study.

MRID 00041283

Citation: Lightkep, G.E. (1980) Teratogenic Potential of CGA-24705 in New Zealand White Rabbits Segment II Evaluation. Argus Research Laboratories, Inc., Horsham, PA. Argus Project 203-001, July 16, 1980. MRID No. 00041283. Unpublished.

EXECUTIVE SUMMARY:

In a prenatal developmental toxicity study (MRID 00041283), CGA-24705 (metolachlor) (95.4% a.i.) in 0.75% aqueous hydroxy methylcellulose was administered by gavage (10 ml/kg) to 16 pregnant New Zealand White rabbits/group from gestation days (GD) 6 through 18, inclusive, at dose levels of 0, 36, 120 or 360 mg/kg/day. The animals were sacrificed on GD 30 and the fetuses examined for evidence of developmental effects.

One doe at 36 mg/kg/day and another at 360 mg/kg/day died on GDs 24 and 29, respectively. The cause of death in both animals was attributed to persistent anorexia. Two rabbits aborted, one at 120

mg/kg/day (GD 25) and another at 360 mg/kg/day (GD 17). The high-dose animal had persistent anorexia. One rabbit in each group delivered prior to GD 30; the control, low- and high-dose animals on GD 29 and the mid-dose animal on GD 30. There was a treatment-related increase in the incidence of persistent anorexia in the does treated at 360 mg/kg/day, which was defined as less than one-half of the daily food allotment consumed. However, food consumption data were not provided to support this finding. There was a treatment-related decrease in body weight gain in the 360 mg/kg/day group for GD 6-18 (-0.16 kg vs +0.04 kg in controls; $p<0.01$) and GD 6-30 (-0.01kg vs +0.03 kg in controls). There was no treatment-related increase in gross pathological findings in maternal animals at necropsy.

No treatment-related increase in external, visceral or skeletal developmental effects was observed.

The maternal toxicity LOAEL was 360 mg/kg/day based on an increased incidence of clinical observations (persistent anorexia) and decreased body weight gain. The NOAEL was 120 mg/kg/day.

The developmental toxicity LOAEL was not established. The NOAEL was 360 mg/kg/day.

The study is classified as **Acceptable/guideline** and **satisfies** the guideline requirements for a prenatal developmental toxicity study in rabbits (83-3b; OPPTS 870.3700).

MRID 00080897

Citation: Page, J.G. (1981) Two-Generation Reproduction Study in Albino Rats with Metolachlor Technical. Toxigenics, Decatur, IL. Study Number 450-0272, August 31, 1981. MRID No. 00080897. Unpublished.

EXECUTIVE SUMMARY:

In a two-generation reproduction study (MRID 00080897), metolachlor (95.4% a.i.) was administered in the diet to two consecutive generations of 15 male/30 female CD albino rats at dose levels of 0, 30, 300 or 1000 ppm (F_0 males: 0, 2.4, 23.5 and 75.8 mg/kg/day; F_0 females: 0, 2.5, 26.0 and 85.7 mg/kg/day; F_1 males: 0, 2.3, 23.7 and 76.6 mg/kg/day; F_1 females: 0, 2.6, 25.7 and 84.5 mg/kg/day).

There were no deaths in the F_0 generation. Two females of the F_1 generation died during the pre-mating period, one in the 300 ppm group at 32 days and the other in the 1000 ppm group at 52 days. One female in the 300 ppm group was found dead on gestation day 19 and a control group female was sacrificed in a moribund condition on lactation day 1. Based on necropsy examinations, none of the deaths was treatment-related. There were no treatment-related clinical signs of toxicity in either generation. Body weight, body weight gain and food consumption were unaffected in the F_0 generation. In the F_1 generation, food consumption was significantly decreased in females of the 1000 ppm group at several timepoints; however, there was no effect on body weight/body weight

gain. Therefore, this finding was not considered toxicologically significant. There were no treatment-related effects on organ weights or gross/microscopic necropsy examinations in either generation.

There was no evidence of a treatment-related effect on any of the reproductive parameters for either generation. Offspring body weight was significantly decreased in the F_1 litter on lactation days 14 and 21 (91- 96% of control value) and in the F_2 litter on lactation days 4, 7, 14 and 21 (92 - 95% of control value). Although the magnitude of the decrease is small, the finding is regarded as toxicologically significant.

The parental toxicity LOAEL was not established. The NOAEL was 1000 ppm (F_0 males/females: 75.8/85.7 mg/kg/day; F_1males/females: 76.6/84.5 mg/kg/day).

The reproductive toxicity LOAEL was not established. The NOAEL was 1000 ppm (F_0 males/females: 75.8/85.7 mg/kg/day; F_1males/females: 76.6/84.5 mg/kg/day).

The offspring LOAEL was conservatively established at 1000 ppm (F_0 males/females: 75.8/85.7 mg/kg/day; F_1males/females: 76.6/84.5 mg/kg/day) based on decreased body weight in F_1 and F_2 litters . The NOAEL is 300 ppm (F_0 males/females: 23.5/ 26.0 mg/kg/day; F_1males/females: 23.7/25.7 mg/kg/day).

The study is classified as **Acceptable/guideline** and **satisfies** the guideline requirements for a multi-generation reproduction study in rats (83-4; OPPTS 870.3800).

MRID 00117597

Citation: Tisdel, M. (1982) Carcinogenicity Study with Metolachlor in Albino Mice. Hazleton-Raltech, Inc., Madison, WI. Study Number 79020, August 13, 1982. MRID No. 00117597. Unpublished.

EXECUTIVE SUMMARY:

In a carcinogenicity study (MRID 00117597), metolachlor (reported to be 95% a.i.) was administered in the diet to 68 CD-1 mice/sex/group at doses of 0, 300, 1000 or 3000 ppm (0, 45, 150 or 450 mg/kg/day, based on 1ppm equals 0.150 mg/kg/day). Eight mice/sex/group were sacrificed at 12 and 18 months.

High dose females had a significant increased mortality rate due to a number of deaths during the first few weeks of treatment (control: 24/52; high dose females: 34/52 at termination) . Although the deaths were possibly attributable to a viral infection, the contribution of the test material can't be dismissed. Body weight was statistically significantly decreased (91-95% of control value) throughout the study in the 3000 ppm males and during the latter half of the study in the 3000 ppm females (93-95%). Body weight gain was consistently decreased in the 3000 ppm males (48-88%) and females (59-86%). Food consumption was comparable between treated and control groups until

week 90 of treatment, at which time the 3000 ppm males consumed 10% less than controls. The decrease was statistically significant at weeks 98, 102 and 104. There was no significant effect on female food consumption. There was no evidence of a treatment-related effect on hematology or clinical chemistry parameters. Organ weight was not affected except for a dose-related decrease in the absolute and relative weight of the seminal vesicles of males which was statistically significant at the high dose. However, there was no effect on testes weight and no accompanying histological changes in the seminal vesicles; therefore, the toxicological significance of the finding is questionable. There were no treatment-related microscopic changes. There was no treatment-related increase in tumor incidence in the study.

The LOAEL was 3000 ppm (450 mg/kg/day) based on possible treatment-related deaths in females and decreased body weight/body weight gain in males and females. The NOAEL was 1000 ppm (150 mg/kg/day).

The HED Cancer Peer Review Committee evaluated the carcinogenic potential of metolachlor on several occasions. At the 4th Committee meeting on July 27, 1994, it was metolachlor was classified as a Group C, possible human carcinogen based on liver tumors in the rat with risk quantitated using a Margin of Exposure approach.

The study is classified as **acceptable/guideline** and **satisfies** the guideline requirements for a carcinogenicity toxicity study in mice (83-5; OPPTS 870.4200).

MRID 00129377

Citation: Tisdel, M. (1983) Two-year Chronic Oral Toxicity and Oncogenicity Study with Metolachlor in Albino Rats. Hazleton-Raltech, Inc., Madison, WI. Study Number 80030, May 2, 1983. MRID No. 00129377. Unpublished.

EXECUTIVE SUMMARY:

In a chronic toxicity/carcinogenicity study (MRID 00129377), metolachlor (95.3% a.i.) was administered in the diet to 60 CD-Crl:CD (SD)BR albino rats/sex/group at dose levels of 0, 30, 300 or 3000 ppm (0, 1.5, 15 or 150 mg/kg/day based on 1 ppm in food equals 0.05 mg/kg/day) for two years. An additional 10 rats/sex/group were administered either 0 (control) or 3000 ppm in the diet for 12 months; five rats/sex/group were sacrificed after the treatment and the remaining five/sex/group were allowed to recover for four weeks and then sacrificed.

This summary applies only to the chronic toxicity portion of the study. The HED Cancer Peer Review Committee evaluated the carcinogenic potential of metolachlor on several occasions. At the 4th Committee meeting on July 27, 1994, metolachlor was classified as a Group C, possible human carcinogen based on liver tumors in rats with risk quantitated using a Margin of Exposure approach.

Comparable mortality rates were observed in the treated and control animals. There were no

treatment-related clinical signs of toxicity. Mean body weight gain was slightly decreased in the 3000 ppm females (6 - 17% decrease) throughout the study; the changes were not statistically significant. Mean food consumption was slightly decreased (4 - 9%) in the 3000 ppm females; the decrease was not statistically significant. Absolute, relative and liver-to-brain weight were increased (7%, 13% and 5%, respectively) in the 3000 ppm males. These increases were also observed in the 3000 ppm males after the four-week recovery period. However, the toxicological significance of the finding is questionable as there were no accompanying clinical pathology or histological changes.

The LOAEL was 3000 ppm (150 mg/kg/day) for females based on slightly decreased body weight gain and food consumption. The NOAEL was 300 ppm (15 mg/kg/day) for females. The LOAEL was not established for males. The NOAEL was 3000 ppm (150 mg/kg/day).

The study is classified as **acceptable/guideline** and **satisfies** the guideline requirements for a chronic toxicity study in rats (83-1; OPPTS 870.4100).

MRID 00151941

Citation: Lochry, E.A. (1985) Embryo/Fetal Toxicity and Teratogenic Potential Study of CGA-24705 (FL-841697) Administered Orally via Gavage to Crl:COBS®CD®(SD) BR Presumed Pregnant Rats. Argus Research Laboratories, Inc., Horsham, PA. Argus Project 203-004, August 6, 1985. MRID No. 00151941. Unpublished.

EXECUTIVE SUMMARY:

In a prenatal developmental toxicity study (MRID 00151941), CGA-24705 (metolachlor) (96.4% a.i.) in 0.5% (w/v) aqueous hydroxymethylcellulose was administered by gavage (10 ml/kg) to 25 presumed pregnant Crl:COBS®CD®(SD)BR rats from gestation days (GD) 6 through 15, inclusive, at dose levels of 0, 30, 100, 300 or 1000 mg/kg/day. The animals were sacrificed on GD 20 and the fetuses examined for evidence of developmental effects.

There were four treatment-related deaths [GD 7, 8 and 10 (2 rats)] in animals treated at 1000 mg/kg/day. Clinical signs of toxicity, including clonic and/or toxic convulsions, excessive salivation, urine-stained abdominal fur and/or excessive lacrimation, were observed in animals treated at 1000 mg/kg/day. There was also an increase in excessive salivation in the 300 mg/kg/day group. However, as this effect was most likely due to gastric irritation and there was no other evidence of treatment-related toxicity, the finding is not considered toxicologically significant. Body weight gain was significantly decreased in the 1000 mg/kg/day group during GD 6-16 (83% of control value; $p<0.05$), GD 6-20 (88% of control value; $p<0.05$) and GD 0-20 (88% of control value; $p<0.01$). Food consumption was not affected.

In the 1000 mg/kg/day group, there was a slightly decreased number of implantations per dam (14.6 vs 15.8 in controls), decreased live fetuses/dam (13.8 vs 15.2 in controls) and increased number of

resorptions/dam (0.8 vs 0.5 in controls). There was also a statistically significant decrease ($p<0.05$; 96% of control value) in mean fetal body weight.

The maternal toxicity LOAEL was 1000 mg/kg/day based on an increased incidence of death, clinical signs of toxicity (clonic and/or toxic convulsions, excessive salivation, urine-stained abdominal fur and/or excessive lacrimation) and decreased body weight gain. The NOAEL was 300 mg/kg/day.

The developmental toxicity LOAEL was conservatively established at 1000 mg/kg/day based on slightly decreased number of implantations per dam, decreased number of live fetuses/dam, increased number of resorptions/dam and significant decrease in mean fetal body weight. The NOAEL was 300 mg/kg/day.

The study is classified as **Acceptable/guideline** and **satisfies** the guideline requirements for a prenatal developmental toxicity study in rats (83-3a; OPPTS 870.3700).

MRIDs 40980701, 41164501, 42218601 and 42218602

Citation: Hazellette, J.R. and Arthur, A. T. (1989) Metolachlor Technical, 13/52-Week Oral Toxicity Study in Dogs (MIN 862253). Division of Toxicology/Pathology, CIBA-GEIGY, Summit, NJ and Methpath Laboratories, Rockville, MD, Study Number 862253, January 23, 1989. MRID Nos. 40980701, 41164501, 42218601 and 42218602. Unpublished.

EXECUTIVE SUMMARY:

In a chronic toxicity study (MRIDs 40980701, 41164501, 42218601 and 42218602), metolachlor (97% a.i.) was administered in the diet to Beagle dogs (6/sex/group for control and high dose groups; 4/sex/group for low- and mid-dose groups) at dose levels of 0, 100, 300 or 1000 ppm (males: 0, 3.5, 9.7 and 32.7 mg/kg/day, respectively; females: 0, 3.6, 9.7 and 33.0 mg/kg/day, respectively) for one year. Two dogs of each sex in the control and high-dose group designated as recovery animals were treated for 52 weeks and were then allowed a 4-week recovery period. An additional 4 dogs/sex/group were treated at the same dose levels and sacrificed at 13 weeks.

There were no treatment-related deaths or clinical signs of toxicity. Mean body weight gain was decreased in the 1000 ppm group females, considering both all animals (5-17% decrease) and only those treated for 52 weeks (5-17% decrease). Alkaline phosphatase was significantly increased in the 1000 ppm females at weeks 12, 26 and 40; however, the increase was not considered toxicologically significant due to the small magnitude of the effect and the lack of accompanying necropsy findings.

The LOAEL was 1000 ppm for females (33.0 mg/kg/day) based on decreased body weight gain. The NOAEL was 300 ppm (9.7 mg/kg/day). The LOAEL for males was not established. The NOAEL for males was 1000 ppm (32.7 mg/kg/day).

The study is classified as **Acceptable/guideline** and **satisfies** the guideline requirements for a chronic toxicity study in dogs (83-1; OPPTS 870.4100).

MRID 41833101

Citation: Mastrocco, F., Huber, K., Schiavo, D.M., Hazelette, J.R. and Green, J.D (1987) 21-Day Dermal Toxicity Study in Rabbits. Research Department, Pharmaceuticals Division, CIBA-GEIGY Corporation. Study Number 862012, November 16, 1987. MRID No. 41833101. Unpublished.

EXECUTIVE SUMMARY:

In a 21-day dermal toxicity study (MRID 41833101), metolachlor (96.4% a.i.) was applied topically once daily for 21 days to the intact skin of five New Zealand rabbits/sex/group at doses of 0, 10, 100 or 1000 mg/kg/day.

All animals survived the treatment. There were no treatment-related effects on clinical signs, body weight/body weight gain, food consumption, ophthalmoscopic examinations, hematology or necropsy examinations. Significant increases in total bilirubin were observed only in females treated at 100 mg/kg/day (68% increase) and 1000 mg/kg/day (72% increase). However, these increases were not considered toxicologically significant as there was no other evidence of organ effects at these doses and hyperbilirubinemia has not been reported in other toxicity studies with metolachlor. Absolute and relative liver weight were significantly increased in the 1000 mg/kg/day males and relative kidney weight was significantly increased in 1000 mg/kg/day females. These effects are not considered toxicologically significant as there were no accompanying laboratory or necropsy findings.

There was evidence of skin irritation in all treated groups. Very slight erythema and dry skin were observed in all animals of the 10 mg/kg/day group; one female at this dose had fissuring. With increasing doses, more animals were observed to have fissuring and wrinkling of the skin. On histopathology, hyperkeratosis, parakeratosis, congestion of the dermis, edema and subacute lymphocytic infiltration were reported in some or all of the treated animals.

The systemic LOAEL was not established. The NOAEL was 1000 mg/kg/day (HDT).

The dermal irritation LOAEL was 10 mg/kg/day (LDT) based on very slight erythema, dry skin and fissuring (one animal). The NOAEL was not established.

The study is classified as **acceptable/guideline** and **satisfies** the guideline requirements for a 21-day dermal toxicity study in rabbits (82-2; OPPTS 870.3200).

MRID 41833102

Citation: Murphy, T. (1987). Dermal Absorption of Metolachlor in Rats. CIBA-GEIGY Corporation,

Agricultural Division, Metabolism Department, Greensboro, NC and WIL Research Laboratories, Ashland, Ohio. Laboratory Study No. ABR-87051, August 25, 1987. MRID No. 41833102. Unpublished.

EXECUTIVE SUMMARY:

In a dermal penetration study (MRID 41833102), ^{14}C-CGA 24705 (% a.i. unknown) suspended in deionized water was applied to a 10 cm^2 area of the backs of 4 male Crl:CD®BR rats/group at doses of 0.01, 0.1 or 1.0 mg/cm^2. Each dose group was exposed for either 2, 4, 10 or 24 hours and then the area was washed and the animals sacrificed. Another 4 animals/dose group were treated for either 10 or 24 hours, the skin was washed and they were placed in a metabolism cage for collection of urine and feces. Sacrifice was 72 hours later. The amount of radioactivity in the blood, urine, feces, carcass, skin and cage wash was determined for all animals.

CGA 24705 was rapidly absorbed with significant bioaccumulation. The total percentage of the applied dose which was found in the blood, urine, feces, carcass and cage wash (or absorbed) after 10 hours was 32.93, 20.26 and 6.98 at 0.01, 0.1 and 1.0 mg/cm^2, respectively. The percentage remaining on the skin was 24.66, 20.89 and 12.69 at the respective doses. The total percentage of the applied dose in the blood, urine, feces, carcass and cage wash (or absorbed) after 24 hours was 62.84, 26.85 and 16.15 at 0.01, 0.1 and 1.0 mg/cm^2, respectively. The percentage remaining on the skin was 11.09, 19.14 and 15.49 at the respective doses.

For rats with skin washings at 10 hours and sacrifice 72 hours after washing, the total percentage of the applied dose found in the blood, urine, feces, carcass and cage wash was 50, 38.61 and 15.46 at 0.01, 0.1 and 1.0 mg/cm^2, respectively. The percentage remaining on the skin was 5.30, 3.48 and 3.54 at the respective doses. For rats with skin washings at 24 hours and sacrifice 72 hours after washing, the percentage of the applied dose found in the blood, urine, feces, carcass and cage wash was 67.32, 43.46 and 30.49 at 0.01, 0.1 and 1.0 mg/cm^2, respectively. The percentage remaining on the skin was 3.39, 1.36 and 1.42 at the respective doses.

The study is classified as **Acceptable/guideline** and **satisfies** the guideline requirements for a dermal penetration study in rats (85-3; OPPTS 870.7600).

DATA EVALUATION REPORT

METOLACHLOR OA
(CGA-51202 TECHNICAL)

STUDY TYPE: ACUTE ORAL TOXICITY - RAT [870.1100 (81-1)]
MRID 44929504

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. 00-10A

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Date: JAN 2 6 2000

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Date: JAN 2 6 2000

Quality Assurance:
Lee Ann Wilson, M.A.

Signature: _____
Date: JAN 2 6 2000

Disclaimer

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

Oak Ridge National Laboratory, managed by Lockheed Martin Energy Research Corp. for the U.S. Department of Energy under contract number DE-AC05-96OR22464.

EPA Reviewer: Virginia A. Dobozy, VMD, MPH Virginia A Dobozy, Date 4/11/01
Reregistration Branch I, Health Effects Division (7509C)
EPA Work Assignment Manager: Joycelyn Stewart, PhD Joycelyn Stewart, Date 5/15/2001
Toxicology Branch, Health Effects Division (7509C)

DATA EVALUATION RECORD

STUDY TYPE: Acute Oral Toxicity - Rat; [OPPTS 870.1100]

DP BARCODE: D260000 SUBMISSION CODE: S569354
P.C. CODE: 108801 (Metolachlor) TOX. CHEM. NO.: 188DD

TEST MATERIAL (PURITY): CGA-51202 Technical (100%)

SYNONYMS: not reported

CITATION: Hartmann, H. (1991) CGA-51202: Acute oral toxicity in the rat. Ciba-Geigy Limited, Short-term toxicology, 4332 Stein, Switzerland. Laboratory study identification 911337, Novartis No. 412-91, December 12, 1991. MRID 44929504. Unpublished.

SPONSOR: Novartis Crop Protection, Inc., 410 Swing Road, P.O. Box 18300, Greensboro, NC 27419

EXECUTIVE SUMMARY: In an acute oral toxicity study (MRID 44929504) groups of five male and five female fasted young adult Tif:RAI f (SPF) rats were given a single oral 2000 mg/kg dose of CGA-51202 Technical (Batch No. JD 7069/3, 100%) in 0.5% w/v carboxy-methylcellulose in 0.1% w/v aqueous polysorbate 80 and observed for 14 days.

Piloerection, hunched posture, exophthalmos, dyspnea, reduced activity, and/or abnormal respiratory sounds were noted on all animals. The surviving animals recovered by day 8. One male was killed for humane reasons on day 13. No other animals died during the study. Mean body weight changes were normal. The euthanized male had a dilated or inflated stomach, small intestine, and typhlon. The remaining animals had no deviations from normal morphology at necropsy.

The oral LD_{50} for males, females, and combined was > 2000 mg/kg (Toxicity Category III).

This acute oral study is classified as **Acceptable/Guideline** and **satisfies** the guideline requirements for an acute oral study [870.1100] in the rat.

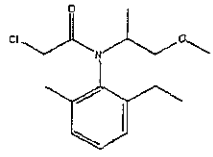
COMPLIANCE: Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: CGA-51202 Technical

Description: beige crystalline
Lot/Batch #: JD 7069/3
Purity: 100%
CAS #: 51218-45-2 (Metolachlor)
Structure:



2. Vehicle and/or positive control

0.5% w/v carboxymethylcellulose in 0.1% w/v aqueous polysorbate 80

3. Test animals

Species: rat
Strain: Tif:RAI f (SPF)
Age and/or weight at dosing: young adult; males and females: 189-249 g; males: 243±3.5 g, females: 197±7.6 g
Source: Ciba-Geigy Limited, Animal Production, 4332 Stein, Switzerland
Acclimation period: at least 5 days
Diet: NAFAG 890 Tox (NAFAG, Gossau/SG, Switzerland), *ad libitum*
Water: *ad libitum*
Housing: Macrolon cages Type 4 (number of animal per cage not reported)
Environmental conditions:
Temperature: 22±2°C
Humidity: 55±10%
Air changes: approximately 15/hour
Photoperiod: 12 hour light/12 hour dark

B. STUDY DESIGN AND METHODS

1. In life dates

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

2. Animal assignment and treatment

Following an overnight fast, groups of five rats/sex were given by gavage a single 2000 mg/kg dose of the test material in 0.5% w/v carboxymethylcellulose in 0.1% w/v aqueous polysorbate 80. The animals were observed for clinical signs of toxicity daily for 14 days. Mortality checks were performed twice daily on week days and once on the weekend. They were weighed on study days 0, 7, and 14. All rats were necropsied.

TABLE 1. Doses, mortality/animals treated

Dose (mg/kg)	Males	Females	Combined
2000	1/5	0/5	1/10

Data taken from p. 13, MRID 44929504.

3. Statistics

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

II. RESULTS AND DISCUSSION

A. MORTALITY

Mortality is given in Table 1. One male was killed for humane reasons on day 13. No other animals died as a result of CGA-51202 Technical toxicity.

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

B. CLINICAL OBSERVATIONS

Piloerection, hunched posture, exophthalmos, dyspnea, reduced activity, abnormal respiratory sounds were noted on all animals. One euthanized male developed a distended abdomen on day 9 and was killed on day 13. The other animals recovered by day 8.

C. BODY WEIGHT

Mean body weight changes were normal.

D. NECROPSY

The euthanized male had dilated or inflated stomach, small intestine, and typhlon. The remaining animals had no deviations from normal morphology.

E. DEFICIENCIES

The study was carried out in 1991 and used only 2000 mg/kg test material for the limit test; however, it is acceptable according the OPPTS 870.1100 guidelines.

DATA EVALUATION REPORT

METOLACHLOR OA
(CGA-51202 TECHNICAL)

STUDY TYPE: ACUTE DERMAL TOXICITY - RAT [870.1200 (81-2)]
MRID 44929505

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. 00-10B

Primary Reviewer:
Susan Chang, M.S.

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Date: JAN 2 6 2000

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Signature: H.T. Borges
Date: JAN 2 6 2000

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

Signature: Robert H. Ross
Date: JAN 2 6 2000

Quality Assurance:
Lee Ann Wilson, M.A.

Signature: L. A. Wilson
Date: JAN 2 6 2000

Disclaimer

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

Oak Ridge National Laboratory, managed by Lockheed Martin Energy Research Corp. for the U.S. Department of Energy under contract number DE-AC05-96OR22464.

EPA Reviewer: Virginia A. Dobozy, VMD, MPH Virginia A Dobozy, Date 4/11/01
Reregistration Branch I, Health Effects Division (7509C)
EPA Work Assignment Manager: Joycelyn Stewart, PhD Joycelyn Stewart, Date 5/15/2001
Toxicology Branch, Health Effects Division (7509C)

DATA EVALUATION RECORD

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

DP BARCODE: D260000 SUBMISSION CODE: S569354
P.C. CODE: 108801 (Metolachlor) TOX. CHEM. NO.: 188DD

TEST MATERIAL (PURITY): CGA-51202 Technical (100%)

SYNONYMS: not reported

CITATION: Hartmann, H. (1991) CGA-51202: Acute dermal toxicity in the rat. Ciba-Geigy Limited, Short-term toxicology, 4332 Stein, Switzerland. Laboratory study identification 911338, Novartis No. 413-91, December 12, 1991. MRID 44929505. Unpublished.

SPONSOR: Novartis Crop Protection, Inc., 410 Swing Road, P.O. Box 18300, Greensboro, NC 27419

EXECUTIVE SUMMARY: In an acute dermal toxicity study (MRID 44929505) approximately 10% of the body surface area of five male and five female young adult Tif:RAI f (SPF) rats was dermally exposed to 1333 mg/kg CGA-51202 Technical (Batch No. JD 7069/3,100%) diluted with distilled water to create a suspension of 33% (w/w) for 24 hours. The animals were observed for 14 days.

None of the animals died during the study. Piloerection and hunched posture were noted in all animals with recovery within 3 days. The mean body weight changes were normal. The study report states that animals had no deviations from normal morphology at necropsy, but no data were submitted.

The dermal LD_{50} for males, females, and combined was > 1333 mg/kg (Toxicity Category II).

This acute dermal study is classified as **Acceptable/Non-guideline** and does not satisfy the guideline requirements for an acute dermal study [870.1200 (81-2)] in the rat. A limit dose of at least 2000 mg/kg is required by the guideline. However, another study is not required as there are sufficient data for Toxicity Category classification.

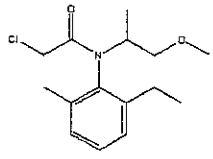
COMPLIANCE: Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: CGA-51202 Technical

Description: beige crystalline
Lot/Batch #: JD 7069/3
Purity: 100%.
CAS #: 51218-45-2 (Metolachlor)
Structure:



2. Vehicle

0.5% w/v carboxymethylcellulose in 0.1% w/v aqueous polysorbate 80

3. Test animals

Species: rat
Strain: Tif:RAI f (SPF)
Age and/or weight at dosing: young adult; males and females: 215-289 g; males: 270±15.7 g, females: 223±7.6 g
Source: Ciba-Geigy Limited, Animal Production, 4332 Stein, Switzerland
Acclimation period: at least 5 days
Diet: NAFAG 890 Tox (NAFAG, Gossau/SG, Switzerland), *ad libitum*
Water: *ad libitum*
Housing: individually in Macrolon cages type 3
Environmental conditions:
Temperature: 22±2°C
Humidity: 55±10%
Air changes: approximately 15/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 1333 mg/kg dose of 33% w/w CGA-51202 Technical in 0.5% w/v carboxymethylcellulose in 0.1% w/v aqueous polysorbate 80 applied to a shaved area (approximately 10% of the body surface) on the back. The study report states that due to the high adsorption of the test material, it was necessary to dilute the test suspension to 33% (w/w). As the limit application volume was 4 ml/kg body weight, the highest dose achievable was 1333 mg/kg.The application site was covered with a gauze-lined semiocclusive dressing and fastened with an adhesive elastic bandage. The covering was removed 24 hours later and the site cleaned with lukewarm water. The animals were observed for clinical signs of toxicity daily for 14 days. Mortality was checked twice daily on week days and once on weekends. The animals were weighed prior to test material application, and on study days 7 and 14. All rats were sacrificed and necropsied.

TABLE 1. Doses, mortality/animals treated

Dose (mg/kg)	Males	Females	Combined
1333	0/5	0/5	0/10

Data taken from p. 13, MRID 44929505.

3. Statistics

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

II. RESULTS AND DISCUSSION

A. MORTALITY

Mortality is given in Table 1. None of the rats died during the study.

The dermal LD_{50} for males, females, and combined was > 1333 mg/kg. This places CGA-51202 Technical in TOXICITY CATEGORY II.

B. CLINICAL OBSERVATIONS

Piloerection and hunched posture were noted from all animals with recovery within 3 days.

C. BODY WEIGHT

The mean body weight changes were normal.

D. NECROPSY

The study report states that animals had no deviations from normal morphology, although no data were submitted.

E. DEFICIENCIES

The study used 1333 mg/kg test material for limit test; however, a limit dose of 2000 mg/kg is required by the guideline.

DATA EVALUATION REPORT

METOLACHLOR OA
(CGA-51202 TECHNICAL)

STUDY TYPE: PRIMARY EYE IRRITATION - RABBIT [870.2400 (81-4)]
MRID 44929506

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. 00-10C

Primary Reviewer:
Susan Chang, M.S.

Signature: S. Chang
Date: JAN 2 6 2000

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

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Date: JAN 2 6 2000

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

Signature: Robert H. Ross
Date: JAN 2 6 2000

Quality Assurance:
Lee Ann Wilson, M.A.

Signature: L. A. Wilson
Date: JAN 2 6 2000

Disclaimer

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

Oak Ridge National Laboratory, managed by Lockheed Martin Energy Research Corp. for the U.S. Department of Energy under contract number DE-AC05-96OR22464.

EPA Reviewer: Virginia A. Dobozy, VMD, MPH Virginia A Dobozy, Date 4/11/01
Reregistration Branch I, Health Effects Division (7509C)
EPA Work Assignment Manager: Joycelyn Stewart, PhD Joycelyn Stewart, Date 5/15/2001
Toxicology Branch, Health Effects Division (7509C)

DATA EVALUATION RECORD

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

DP BARCODE: D260000 SUBMISSION CODE: S569354
P.C. CODE: 108801 (Metolachlor) TOX. CHEM. NO.: 188DD

TEST MATERIAL (PURITY): CGA-51202 Technical (100%)

SYNONYMS: not reported

CITATION: Hagemann (1992) CGA-51202: Acute eye irritation/corrosion study in the rabbit. Ciba-Geigy Limited, Short-term toxicology, 4332 Stein, Switzerland. Laboratory study identification 911339, Novartis No. 414-91, March 27, 1992. MRID 44929506. Unpublished.

SPONSOR: Novartis Crop Protection, Inc., 410 Swing Road, P.O. Box 18300, Greensboro, NC 27419

EXECUTIVE SUMMARY: In a primary eye irritation study (MRID 44929506) 38 mg (0.1 mL weight equivalent) of CGA-51202 Technical (Batch No. JD 7069/3, 100%) was instilled into the conjunctival sac of the left eye of three male rabbits and the eye lids held together for approximately 1 second. The contralateral eye of all rabbits served as control. The eyes were scored for ocular irritation according to the OECD scoring system 1, 24, 48, and 72 hours and days 7, 10, 14, 17, and 21 after test material instillation.

Corneal opacity was found on 3/3 rabbits one hour after test material instillation that persisted through day 10 with resolution on two rabbits by day 14 and on one rabbit by day 21. Iritis was noted on 3/3 rabbits one hour after test material instillation that persisted through 72 hours with resolution on two rabbits by day 7 and on one rabbit by day 10. All rabbits had evidence of conjunctival chemosis at one hour post-instillation, which cleared by either 7, 10 or 14 days. All rabbits had conjunctival redness by one hour post-instillation, which cleared by either 17 or 21 days.

In this study, CGA-51202 Technical was a severe irritant and is in TOXICITY CATEGORY II for primary eye irritation.

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

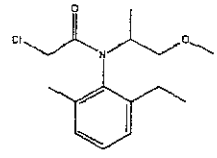
COMPLIANCE: Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: CGA-51202 Technical

   Description: beige crystalline
   Lot/Batch #: JD 7069/3
   Purity: 100%
   CAS #: 51218-45-2 (Metolachlor)
   Structure:



2. Vehicle

   None

3. Test animals

   Species: rabbit
   Strain: New Zealand White (Chbb:NZW)
   Age and weight at dosing: age not reported, but assumed to be young adults according to the body weights; males: 2480-2630 g
   Source: Dr. K. Thomae GMBH, Chemisch-pharmazeutische Fabrik, D-7950 Biberach, Riss
   Acclimation period: at least 5 days
   Diet: NAFAG No. 814 (Gossau, Switzerland), *ad libitum*
   Water: fresh water, *ad libitum*
   Housing: individually in metal cages
   Environmental conditions:
   Temperature: 20±3°C
   Humidity: 30-70%
   Air changes: not reported
   Photoperiod: 12 hour light/dark

B. STUDY DESIGN AND METHODS

1. In life dates

Start: November 26, 1991; end: February 25, 1992

2. Animal assignment and treatment

The test material (0.1 mL weighing 38 mg) was instilled into the conjunctival sac of the left eye of three male 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 and 7, 14, 17, and 21 days after instillation according to the OECD scoring system. The eyes were examined using a slit-lamp.

3. Body weight - body weights were measured at the start of the test and on days 3, 7, 14 and 21.

II. RESULTS AND DISCUSSION

A. Corneal opacity was found on 3/3 rabbits one hour after test material instillation that persisted through day 10 with resolution on two rabbits by day 14 and on one rabbit by day 21. This rabbit had a corroded conjunctiva with hemorrhagic parts on day 3, minimal corneal bulging on days 7-14, and vascularization of the lower marginal zone of the cornea on days 7-17. Iritis was noted on 3/3 rabbits one hour after test material instillation that persisted through 72 hours with resolution on two rabbits by day 7 and on one rabbit by day 10. All rabbits had evidence of conjunctival chemosis at one hour post-instillation, which cleared by either 7, 10 or 14 days. All rabbits had conjunctival redness by one hour post-instillation, which cleared by either 17 or 21 days.

This classifies the test material as a severe irritant. CGA-51202 Technical is in TOXICITY CATEGORY II.

B. Body weight - all animals gained weight during the study.

B. DEFICIENCIES

The study author did not state why the experiment took 3 months to complete (November 26, 1991 to February 25, 1992).

The study report stated that the ocular reactions were according to the OECD scoring system (Appendix 1) and the irritant/corrosive potency of the test material was classified according to the Commission Directive 83/467/EEC (Appendix 2). The reviewer did not find the Appendices in the study report.

The frequency of air changes of the animal room was not reported. This would not affect the study results.

DATA EVALUATION REPORT

METOLACHLOR OA
(CGA-51202 TECHNICAL)

STUDY TYPE: PRIMARY DERMAL IRRITATION - RABBIT [870.2500 (81-5)]
MRID 44929507

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. 00-10D

Primary Reviewer:
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Signature: S. Chang
Date: JAN 2 6 2000

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

Signature: Robert H. Ross
Date: JAN 2 6 2000

Quality Assurance:
Lee Ann Wilson, M.A.

Signature: L. A. Wilson
Date: JAN 2 6 2000

Disclaimer

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

Oak Ridge National Laboratory, managed by Lockheed Martin Energy Research Corp. for the U.S. Department of Energy under contract number DE-AC05-96OR22464.

EPA Reviewer: Virginia A. Dobozy, VMD, MPH Virginia A Dobozy, Date 4/11/01
Reregistration Branch I, Health Effects Division (7509C)
EPA Work Assignment Manager: Joycelyn Stewart, PhD Joycelyn Stewart, Date 5/15/2001
Toxicology Branch, Health Effects Division (7509C)

DATA EVALUATION RECORD

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

DP BARCODE: D260000 SUBMISSION CODE: S569354
P.C. CODE: 108801 (Metolachlor) TOX. CHEM. NO.: 188DD

TEST MATERIAL (PURITY): CGA-51202 Technical (100%)

SYNONYMS: not reported

CITATION: Hagemann (1991) CGA-51202: Acute dermal irritation/corrosion in the rabbit. Ciba-Geigy Limited, Short-term toxicology, 4332 Stein, Switzerland. Laboratory study identification 911340, Novartis No. 415-91, December 11, 1991. MRID 44929507. Unpublished.

SPONSOR: Novartis Crop Protection, Inc., 410 Swing Road, P.O. Box 18300, Greensboro, NC 27419

EXECUTIVE SUMMARY: In a primary dermal irritation study (MRID 44929507) three male adult New Zealand white rabbits were dermally exposed to 0.5 g CGA-51202 Technical (Batch No. JD 7069/3, 100%) on a gauze patch moistened with 0.5% w/v carboxymethylcellulose in 0.1% w/v aqueous polysorbate 80 for 4 hours on the flank of the animals. The application sites were scored for erythema and edema 1, 24, 48, and 72 hours after patch removal using the OECD scoring system.

Very slight erythema was noted on 2/3 rabbits one hour following patch removal with resolution by 24-hours.

In this study, CGA-51202 Technical was essentially nonirritating and is in TOXICITY CATEGORY IV for primary dermal irritation.

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

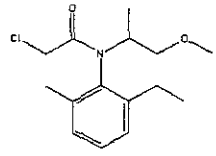
COMPLIANCE: Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: CGA-51202 Technical

Description: beige crystalline
Lot/Batch #: JD 7069/3
Purity: 100%.
CAS #: 51218-45-2 (Metolachlor)
Structure:



2. Vehicle

0.5% w/v carboxymethylcellulose in 0.1% w/v aqueous polysorbate 80

3. Test animals

Species: rabbit
Strain: New Zealand White
Age and weight at dosing: age not reported, but assumed to be young adults according the body weights; males: 2690-2960 g
Source: Dr. K. Thomae GMBH, Chemisch-pharmazeutische Fabrik, D-7950 Biberach, Riss
Acclimation period: at least 5 days
Diet: NAFAG No. 814 (Gossau, Switzerland), *ad libitum*
Water: fresh water, *ad libitum*
Housing: individually in metal cages
Environmental conditions:
Temperature: 20±3°C
Humidity: 30-70%
Air changes: not reported
Photoperiod: 12 hour light/dark

B. STUDY DESIGN AND METHODS

1. In life dates

Start: November 19, 1991; end: November 22, 1991

2. Animal assignment and treatment

Three male animals were given a single 0.5 g dose of CGA-51202 Technical applied to an approximately 12-16 cm^2 gauze patch (moistened with 0.5% w/v carboxymethylcellulose in 0.1% w/v aqueous polysorbate 80) and placed on the shaved right flank. A gauze patch moistened with 0.5% w/v carboxymethylcellulose in 0.1% w/v aqueous polysorbate 80 was placed on the contralateral flank as a control. The patches were loosely covered with aluminum foil and held in place with adhesive tape. The dressing was removed after four hours. The site was scored for erythema and edema 1, 24, 48, and 72 hours after patch removal according to the OECD scoring system.

II. RESULTS AND DISCUSSION

A. Very slight erythema was noted on 2/3 rabbits one hour following patch removal with resolution by 24 hours.

CGA-51202 Technical was essentially nonirritating and is in TOXICITY CATEGORY IV.

B. DEFICIENCIES

The frequency of air changes of the animal room was not reported. This would not affect the study results.

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DATA EVALUATION REPORT

METOLACHLOR OA
(CGA-51202 TECHNICAL)

STUDY TYPE: DERMAL SENSITIZATION - GUINEA PIG [870.2600 (81-6)]
MRID 44929508

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. 00-10E

Primary Reviewer:
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Signature: Susan Chang
Date: JAN 2 6 2000

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Signature: H.T Borges
Date: JAN 2 6 2000

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

Signature: Robert H Ross
Date: JAN 2 6 2000

Quality Assurance:
Lee Ann Wilson, M.A.

Signature: L. A. Wilson
Date: JAN 2 6 2000

Disclaimer

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

Oak Ridge National Laboratory, managed by Lockheed Martin Energy Research Corp. for the U.S. Department of Energy under contract number DE-AC05-96OR22464.

EPA Reviewer: Virginia A. Dobozy, VMD, MPH Virginia A Dobozy, Date 4/11/01
Reregistration Branch I, Health Effects Division (7509C)
EPA Work Assignment Manager: Joycelyn Stewart, PhD Joycelyn Stewart, Date 5/15/2001
Toxicology Branch, Health Effects Division (7509C)

DATA EVALUATION RECORD

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

DP BARCODE: D260000 SUBMISSION CODE: S569354
P.C. CODE: 108801 (Metolachlor) TOX. CHEM. NO.: 188DD

TEST MATERIAL (PURITY): CGA-51202 Technical (100%)

SYNONYMS: not reported

CITATION: Hagemann (1992) CGA-51202: Skin sensitization test in the guinea pig (Optimization test). Ciba-Geigy Limited, Short-term toxicology, 4332 Stein, Switzerland. Laboratory study identification 911341, Novartis No. 526-92, May 18, 1992. MRID 44929508. Unpublished.

SPONSOR: Novartis Crop Protection, Inc., 410 Swing Road, P.O. Box 18300, Greensboro, NC 27419

EXECUTIVE SUMMARY: In a dermal sensitization study (MRID 44929508) with CGA-51202 Technical (Batch No. JD 7069/3, 100%), 40 young adult male and female guinea pigs were tested using the Optimization Test.

Following ten intradermal inductions with a 0.1% (w/w) solution of the test material, 5/20 test animals showed positive reactions after intradermal challenge; the control group had no positive reactions after intradermal challenge. Twenty-four hours after epidermal challenge, 3/20 animals showed positive reactions and 11/20 animals showed positive reactions at 48 hours. The sites of the test animals treated with the vehicle did not have any positive reaction. One animal in the control group treated with the test material had a positive reaction 48 hours after epidermal challenge. No animals in the control group treated with the vehicle showed positive reactions. The historical control data demonstrated that 1-chlor-2,4-dinitrophenol positive control and vehicle control animals responded appropriately.

In this study, CGA-51202 Technical was a dermal sensitizer.

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

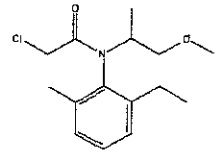
COMPLIANCE: Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: CGA-51202 Technical

Description: beige crystalline
Lot/Batch #: JD 7069/3
Purity: 100%.
CAS #: 51218-45-2 (Metolachlor)
Structure:



2. Vehicle and positive control

Vehicle: physiological saline; positive control: 1-chlor-2,4-dinitrophenol

3. Test animals

Species: guinea pig
Strain: Pirbright White Strain (Tif:DHP)
Age and weight at start of treatment: age not reported; but assumes to be adult according to the body weights; males: 360-432 g; females: 360-430 g
Source: Ciba-Geigy Limited, Animal Production, 4332 Stein, Switzerland
Acclimation period: 5 days
Diet: NAFAG No. 845 (Gossau/SG, Switzerland), *ad libitum*
Water: fresh water, *ad libitum*
Housing: individually in Macrolon type 3 cages
Environmental conditions:
Temperature: 22±3°C
Humidity: 30-70%
Air changes: not reported
Photoperiod: 12 hour light/dark

B. STUDY DESIGN AND METHODS

1. In life dates

Start: January 13, 1992; end: March 5, 1992

2. Animal assignment and treatment

The animals were induced and challenged according to the Optimization Test. Induction: Ten male and ten female animals received one injection on the shaved right flank and back every other day (except weekends) for a total of 10 intradermal injections of 0.1 mL of freshly prepared 0.1% w/w test material solution in physiological saline. Ten male and ten female animals were treated with the vehicle alone as controls. During the second and third week of induction the test material was incorporated w/v in a mixture of the normal vehicle with complete Bacto adjuvant (vehicle : adjuvant - 1:1 v/v, 0.1 mL per injection). No treatments were performed during weeks 4 and 5. Intradermal challenge: The animals were challenged by injection of freshly prepared solution of 0.1% w/w test material in physiological saline on the left flank during week 6. The control group was treated with the vehicle alone. The sites were evaluated 24 hours post exposure. No treatments were performed during week 7. Epidermal challenge: During week 8, the animals were challenged epidermally under occlusive dressings with 10% w/w test material in vaseline and vaseline alone. The control group was treated with the vehicle as well as with the test material to check the maximum subirritation concentration of the test material in adjuvant treated animals. The dressing was left in place for 24 hours. Reactions were scored 24 and 48 hours after inductions and challenge.

II. RESULTS AND DISCUSSION

A. INDUCTION REACTIONS AND DURATION

No data were reported.

B. CHALLENGE REACTIONS AND DURATION

Five out of twenty test animals showed positive reactions after intradermal challenge as compared to 0/20 control animals. Twenty-four hours after epidermal challenge, 3/20 animals showed positive reactions and eleven animals showed positive reactions at 48 hours. The sites of the test animals treated with the vehicle did not have any positive reactions. One animal in the control group treated with the test material had a positive reaction 48 hours after epidermal challenge. No animals in the control group treated with the vehicle showed positive reaction.

CGA-51202 Technical was a dermal sensitizer.

C. POSITIVE CONTROL

The study report included a positive control study which was completed June 13, 1991, approximately 9 months prior to the current study. The 1-chlor-2,4-dinitrophenol positive control and vehicle control animals responded appropriately.

D. ADDITIONAL TESTING

Although the positive control study was beyond six months of the current study, the test material was a sensitizer. There is no need for additional studies.

E. DEFICIENCIES

The positive control study was not done within six months of the current study. The frequency of air changes of the animal room was not reported. These would not affect the study results.

DATA EVALUATION REPORT

CGA-51202
(METOCHLOR OA) (DEGREDATE OF METOCHLOR)

STUDY TYPE: SUBCHRONIC ORAL TOXICITY FEEDING - RAT [OPPTS 870.3100 (82-1)]
MRID 44929509

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. 00-10F

Primary Reviewer: Cheryl B. Bast, Ph.D., D.A.B.T.	Signature: Date:	Cheryl B Bast JAN 12 2000
Secondary Reviewers: H. Tim Borges, Ph.D., D.A.B.T., MT(ASCP)	Signature: Date:	H.T Borges JAN 12 2000
Robert H. Ross, M.S., Group Leader	Signature: Date:	Robert H Ross JAN 12 2000
Quality Assurance: Lee Ann Wilson, M.A.	Signature: Date:	L. A. Wilson JAN 12 2000

Disclaimer

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

Oak Ridge National Laboratory, managed by Lockheed Martin Energy Research Corp. for the U.S. Department of Energy under contract number DE-AC05-96OR22464.

EPA Reviewer: Virginia A. Dobozy, VMD, MPH Virginia A Dobozy, Date 7/5/01
Reregistration Branch I, Health Effects Division (7509C)
EPA Work Assignment Manager: Joycelyn Stewart, PhD Joycelyn Stewart, Date 7/11/2001
Toxicology Branch, Health Effects Division (7509C)

DATA EVALUATION RECORD

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

DP BARCODE: D260000
P.C. CODE: 108801 (parent)
SUBMISSION CODE: S569354
TOX. CHEM. NO.: 188DD

TEST MATERIAL (PURITY): CGA-51202 (Metochlor OA, degredate of metochlor, 100% a.i.)

SYNONYMS: Not provided

CITATION: Schneider, M. (1992) CGA-51202: Final Report. 3-Month oral toxicity study in rats (Administration in food). CIBA-GEIGY Limited, Short/Long-term Toxicology, 4332 Stein, Switzerland. Laboratory Study ID: 911344, July 23, 1992. MRID 44929509. Unpublished.

SPONSOR: Novartis Crop Protection, Inc., 410 Swing Road, Post Office Box 18300, Greensboro, NC 27419.

EXECUTIVE SUMMARY: In a subchronic oral feeding study, (MRID 44929509), CGA-51202 technical (100% a.i.; batch No. JD 7069/3) was fed to groups of 10 male and 10 female albino rats at dose levels of 0, 300, 1000, or 15,000 ppm for 3 months. The average achieved doses for the corresponding groups were 0, 18.7, 62.1, and 1000 mg/kg bodyweight for males, and 0, 20.6, 67.3, and 1020 mg/kg for females.

All animals survived to study termination and no treatment-related clinical signs were observed. There were no treatment-related effects on body weight, food consumption, ophthalmoscopic parameters, or urinalysis. Platelet counts were decreased 16% ($p<0.01$) in high-dose males. Total protein in high-dose males (5% decrease, $p<0.01$) and females (4% decrease, N.S.) was slightly decreased due to decreased globulin in males and decreased albumin and globulin fractions in females. These effects were not considered biologically significant. There were no treatment-related organ weight effects or macroscopic or microscopic lesions. **Under the conditions of this study, the NOAEL is 15,000 ppm in the diet (1000 mg/kg for males, 1020 mg/kg for females, limit dose) based on no biologically significant effects. A LOAEL was not identified.**

This subchronic toxicity study in rats (82-1) is classified as **Acceptable/Guideline.** It satisfies the guideline requirement for a subchronic dietary toxicity study in rodents.

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

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: CGA- 51202 technical

Description: beige, crystalline
Lot/Batch #: JD 7069/3
Purity: 100%
Stability of compound: study report states "September, 1995"
CAS #: not provided

2. Vehicle and/or positive control

Vehicle: Diet, Nafag No. 890 Tox.

3. Test animals

Species: albino rat
Strain: Tif: RAIf (SPF) hybrids of RII/1 x RII/2
Age and weight at study initiation: approximately 5-6 weeks old; males: 112.5-132.0 g; females: 105.2-124.3 g
Source: CIBA-GEIGY Limited, 4332 Stein, Switzerland
Housing: housed 5/macrolon type 4 cage with wire mesh tops
Diet: Nafag No. 890 Tox., *ad libitum*
Water: drinking water (tap) available, *ad libitum*
Environmental conditions:
Temperature: 22 ± 2°C
Humidity: 55 ± 10
Air changes: 16-20 changes/hour
Photoperiod: 12 hours light/dark cycle
Acclimation period: 7 days

B. STUDY DESIGN

1. In life dates

Start: December 10, 1991; end: March 11, 1992

2. Animal assignment

Rats were assigned randomly to the study using a computer-generated program. The study design is shown in Table 1.

TABLE 1. Study design					
Test group	Conc. in diet (ppm)	Achieved dose (mg/kg)		Number of animals	
		Male	Female	Male	Female
Control	0	0	0	10	10
low-dose	300	18.7	20.6	10	10
mid-dose	1000	62.1	67.3	10	10
high-dose	15,000	1000	1020	10	10

Data taken from pp. 17 and 34-35, MRID 44929509.

3. Test material preparation and analysis

CGA-51202 technical was weighed and appropriate amounts were homogeneously mixed with pulverized food. The food was pelleted by adding ~25% drinking water to ensure necessary quality. Pellets were then air dried. Test diets were prepared at monthly intervals and stored in stainless steel containers at room temperature. Homogeneity analysis was performed by HPLC on food samples containing the test article at concentrations of 100, 1000 and 15000 ppm from three different segments of feed preparation (beginning, middle, end). Stability analyses of test diets were performed by HPLC on days 0 and 35. Concentration analyses were performed by HPLC on test diets prepared on December 4, 1991 and January 27, 1992.

Results -
Homogeneity analysis: The range of the test compound concentration from the sample's beginning, middle, and end varied in the range of -3% to +4% of the mean concentration.

Stability analysis: The CGA 51202 was found to be stable in rodent feed at room temperature over a period of 5 weeks. Percentages of the initial values after 35 days were: 102.6% at 100 ppm, 99.2% at 1000 ppm, and 97.7% at 15000 ppm.

Concentration analysis: CGA 51202 concentration ranges were 86.6-92.3% (300 ppm), 89.5-89.6% (1000 ppm), and 88.3-90.6% (15000 ppm). No test compound was detected in the control sample.

The analytical data indicated that the mixing procedure was adequate and that the variance between nominal and actual dosage to the animals was acceptable.

4. Statistics

"For each time point and parameter an univariate statistical analysis was performed. Nonparametric methods were applied, to allow for non normal as well as normal data

distribution. Each treated group was compared to the control group by Lepage's two-sample test and tested for increasing or decreasing trends from control up to the respective dose group by Jonckheere's test for ordered alternatives."

C. METHODS

1. Observations

All animals were observed twice daily (morning and afternoon) for mortality and signs of overt toxicity. All animals received a detailed physical at least weekly.

2. Body weight

All animals were weighed weekly beginning during the acclimation period.

3. Food consumption and compound intake

Food consumption was recorded weekly. Food consumption ratios were calculated as the mean of individual weekly ratios as follows:

$$\frac{\text{Weekly food consumption (g)}}{\text{midweek bodyweight (g)}} \times \frac{1000}{7} = \text{g food / kg bodyweight / day}$$

Compound intake was calculated (mg/kg/day) for corresponding food consumption intervals as follows:

4. Ophthalmoscopic examination

Ophthalmologic examinations were conducted on control and high-dose animals using an ophthalmoscope. Examinations were performed prior to study initiation (day -6) and after 85 days of treatment.

5. Blood was collected from the orbital sinus of ether anesthetized animals at the end of the study. The animals were fasted overnight prior to sample collection. 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*		Reticulocyte count
	Blood clotting measurements*		Heinz body determination
	(Thromboplastin time)		RBC morphology
	(Clotting time)		
x	(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	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)		Triglycerides
	Cholinesterase (ChE)		Serum protein electrophoresis
	Creatine phosphokinase		
	Lactic acid dehydrogenase (LDH)		
x	Serum alanine amino-transferase (also SGPT)*		
x	Serum aspartate amino-transferase (also SGOT)*		
x	Gamma glutamyl transferase (GGT)		
	Glutamate dehydrogenase		

* Required for subchronic studies based on Subdivision F Guidelines

6. Urinalysis*

Urine was collected from individual animals in metabolism cages overnight. Food and water were withheld during the sample collection period. The CHECKED (X) parameters were examined.

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

*Not required for subchronic studies by Subdivision F Guidelines.

7. Sacrifice and pathology

All animals survived the treatment period and were sacrificed under ether anesthesia on schedule by exsanguination. Gross pathological examination was performed on all rats, and the CHECKED (X) tissues were preserved in 4% neutral buffered formalin. The (XX) organs were weighed. The X* organs were embedded in paraplast, sectioned at 3-5 microns, stained with hematoxylin and eosin, and examined microscopically.

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

** = Required for subchronic studies based on Subdivision F Guidelines

$^{+}$ = Organ weight required in subchronic and chronic studies.

^{T} = Required only when toxicity or target organ

^{l} = Cervical spine only

II. RESULTS

A. OBSERVATIONS

1. Toxicity

There were no compound-related clinical observations observed.

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2. Mortality

All animals survived to study termination.

B. BODY WEIGHT

No treatment-related body weight effects were noted. Data are summarized in Table 2.

TABLE 2. Group mean body weights (g) in rats fed CGA51202 for 13 weeks				
Week of study	Exposure concentration (ppm)			
	0	300	1000	15,000
	Males			
-1	122.0	123.6	122.2	122.0
1	184.4	184.6	181.1	179.4
2	248.7	245.3	242.2	240.9
3	309.0	302.2	297.6	302.7
4	353.8	340.6	344.0	344.4
5	384.8	377.5	382.8	381.4
6	416.3	406.4	412.2	410.1
7	444.1	431.2	438.2	434.1
8	468.1	453.1	460.1	458.2
9	485.1	466.7	476.6	474.1
10	503.1	480.4	489.4	481.8
11	515.4	495.5	503.2	492.4
12	526.5	507.4	516.0	504.5
13	539.8	520.9	531.1	518.1
	Females			
-1	114.6	113.9	114.4	114.5
1	154.9	153.5	154.3	152.3
2	181.7	188.0	186.7	185.3
3	205.7	215.7	217.4	210.9
4	234.3	239.6	245.5	237.3
5	249.3	260.3	262.8	254.4
6	257.5	269.9	267.4	268.8
7	269.4	287.1	282.7	278.4
8	283.0	301.9	294.5	288.6
9	288.1	310.6	305.6	293.4
10	294.7	312.4	309.8	297.2
11	300.4	321.8	320.1	306.0
12	305.2	325.3	320.4	312.9
13	312.6	327.9	323.1	312.0

Data taken from pp. 38-39, MRID 44929509.
No Statistical significance was achieved.

C. FOOD CONSUMPTION AND COMPOUND INTAKE

1. Food consumption

No treatment-related, biologically significant food consumption effects were observed. Food consumption was decreased ($p<0.05$) 6.8% in mid-dose males and 9.8% in high-dose males compared to controls during week 1 only. Food consumption was increased ($p<0.05$) 17% in mid-dose females compared to controls during week 3 only.

2. Compound consumption

Animals were offered diets containing the compound *ad libitum* for 90 days. Achieved doses are shown in Table 1.

3. Food consumption ratios

Food consumption ratios were 2.1-11.7% higher than controls in high-dose males from weeks 3 through 13. No other effects were noted.

D. OPHTHALMOSCOPIC EXAMINATION

There were no treatment-related findings.

E. BLOOD WORK

1. Hematology

Platelet counts were decreased ($p<0.01$) 16% in high-dose males compared to controls. This change is not considered biologically relevant.

2. Clinical chemistry

Clinical chemistry parameter effects were noted in high-dose animals. Total protein in high-dose males (5% decrease, $p<0.01$) and females (4% decrease, N.S.) was slightly decreased due to decreased globulin in males and decreased albumin and globulin fractions in females. Other observations ($p<0.01$) included a 23% decrease in total bilirubin and a 22% decrease in cholesterol in high-dose males, and a 21% decrease in alanine amino transferase in high-dose females compared to controls. These effects are not considered biologically relevant as they are all within historical control ranges.

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F. URINALYSIS

No treatment-related effects were noted.

G. SACRIFICE AND PATHOLOGY

1. Organ weight

No biologically significant, treatment-related effects were noted. Mean absolute liver weight was decreased ($p<0.01$) 10-16% in all male treatment groups compared to the control. However, a dose-response was not observed and the author attributes the apparent effect to a comparatively high mean value for the control group (compared to historical controls). The mean relative adrenal weight was increased ($p<0.01$) 6% in high-dose females and 11% in high-dose males compared to controls; however, no corroborative pathology was observed. No other organ weight effects were observed.

2. Gross pathology

There were no treatment-related gross lesions.

3. Microscopic pathology

There were no treatment-related microscopic lesions.

III. DISCUSSION

A. DISCUSSION

All animals survived to study termination and no treatment-related clinical signs were observed. There were no treatment-related effects on body weight, food consumption, opthlamological parameters, or urinalysis. Platelet counts were decreased 16% ($p<0.01$) in high-dose males. Total protein in high-dose males (5% decrease, $p<0.01$) and females (4% decrease, N.S.) was slightly decreased due to decreased globulin in males and decreased albumin and globulin fractions in females. The observed hematology and clinical chemistry changes are not considered biologically relevant since they are within historical control ranges and/or are of small magnitude. There were no treatment-related organ weight effects or macroscopic or microscopic lesions.

It is probable that the minor hematological and clinical chemistry changes in observed high-dose animals are treatment-related, and can be considered a LOEL. However, in light of the small magnitude and biological insignificance of the changes, they do not, in the reviewer's opinion, define a LOAEL. It should be noted that the study author did consider the high dose to be a LOAEL.

Under the conditions of this study, the NOAEL is 15,000 ppm (1000 mg/kg for males, 1020 mg/kg for females, limit dose) based on a lack of biologically significant effects. A LOAEL was not identified.

This subchronic toxicity study in rats [870.3100 (82-1)] is classified as **Acceptable/Guideline.** It satisfies the guideline requirement for a subchronic dietary toxicity study in rodents.

B. STUDY DEFICIENCIES

Although a LOAEL was not determined, CGA-51202 was tested up to the limit dose. A minor deficiency is the lack of histopathological evaluation of the rectum. This does not compromise the study.

DATA EVALUATION REPORT

Metolachlor OA (CGA-51202 TECHNICAL)

STUDY TYPE: DEVELOPMENTAL TOXICITY- RAT [870.3700 (§83-3a)]
MRID 44929510

Prepared for

Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
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Prepared by

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Disclaimer

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

Managed by Lockheed Martin Energy Research, Corp. for the U.S. Department of Energy under Contract No. DE-AC05-96OR22464.

EPA Reviewer: Virginia A. Dobozy, VMD, MPH Virginia A Dobozy Date: 4/10/01
Reregistration Branch I, Health Effects Division (7509C)
EPA Work Assignment Manager: Joycelyn Stewart, PhD Joycelyn E Stewart Date: 4/26/01
Toxicology Branch, Health Effects Division (7509C)

DATA EVALUATION RECORD

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

DP BARCODE: D260000 SUBMISSION CODE: S569354
P.C. CODE: 108801 (parent) TOX. CHEM. NO.: 188DD

TEST MATERIAL (PURITY): CGA-51202 Technical (100% a.i.)

SYNONYMS: none; degradate of metolachlor

CITATION: Marty, J.H. (1992) CGA-51202 technical: Rat oral teratogenicity. Ciba-Geigy Limited, Reproduction Toxicology, 4332 Stein, Switzerland. Laboratory Study No. 911351. November 3, 1992. MRID 44929510. Unpublished.

SPONSOR: Novartis Crop Protection, Inc., 410 Swing Road, Post Office Box 18300, Greensboro, NC 27419

EXECUTIVE SUMMARY: In a developmental toxicity study (MRID 44929510), 24 presumed pregnant Tif: RAI f (SPF) (hybrids of RII/1 × RII/2) rats per group were administered CGA 51202 Technical (100%; Batch No. JD 7069/3) by gavage in 0.5% aqueous sodium carboxy-methylcellulose solution at doses of 0, 10, 100, or 1000 mg/kg/day on gestation days (GD) 6-15, inclusive. Controls were treated with 0.5% sodium carboxymethylcellulose (vehicle). On GD 21, dams were sacrificed, subjected to gross necropsy, and all fetuses examined externally. Approximately one-half of each litter was processed for visceral examination and the remaining one-half was processed for skeletal examination.

One low-dose animal was sacrificed moribund on GD 20 with a urogenital infection. All other animals survived to terminal sacrifice. No clinical signs of toxicity were observed in any animal. Maternal body weights and body weight gains were similar between the treated and control groups throughout the study. Food consumption was not affected by treatment. Maternal necropsy was unremarkable.

Therefore, the maternal toxicity NOAEL is ≥1000 mg/kg/day and the maternal toxicity LOAEL was not identified.

No differences were observed between the treated and control groups for number of corpora lutea, number of implantation sites, live fetuses/dam, pre- and post-implantation losses, fetal body weights, or fetal sex ratios.

No treatment-related external, visceral, or skeletal malformations/variations were observed in any fetus from any group.

The high dose is equivalent to the limit dose for developmental toxicity studies.

Therefore, the developmental toxicity NOAEL is ≥1000 mg/kg/day and the developmental toxicity LOAEL was not identified.

This study is classified as **Acceptable/Guideline** and satisfies the requirements for a developmental toxicity study [870.3700 (§83-3a)] in rats.

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

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material: CGA-51202 Technical

   Description: beige crystals
   Batch No.: JD 7069/3
   Purity: 100% a.i.
   Stability of compound: not stated
   CAS No.: not given
   Structure: not given

2. Vehicle and/or positive control

   A 0.5% aqueous solution of sodium carboxymethylcellulose (CMC, Hercules Powder Company, Pharmacopeia quality, high viscosity, Prod. 7HF) was used as the vehicle and negative control. No positive control was used in this study.

3. Test animals

   Species: rat
   Strain: Tif: RAI f (SPF), hybrids of RII/1 × RII/2
   Age and weight at study initiation: minimum of 8 weeks; 173.7-220.4 g
   Source: Animal Production, WST-455, Ciba-Geigy Limited, 4332 Stein, Switzerland
   Housing: Animals were individually housed in Macrolon cages with wire mesh tops and standardized granulated soft wood bedding material.
   Diet: Pelleted certified standard feed (Nafag No. 890, Tox; Nafag, Naehr- und Futtermittel AG, Gossau, Switzerland) was available *ad libitum*.
   Water: Tap water was available *ad libitum*.
   Environmental conditions:
   Temperature: 22 ± 3°C
   Humidity: 50 ± 20%
   Air changes: about 16/hour
   Photoperiod: 12 hr light/dark
   Acclimation period: at least 7 days between delivery from animal production (in house) and the first day of treatment

B. PROCEDURES AND STUDY DESIGN

This study was designed to assess the developmental toxicity potential of CGA-51202 Technical when administered by gavage to rats on GD 6-15, inclusive.

1. In life dates

Start: April 7, 1992; end: April 28, 1992 (start of necropsy)

2. Mating

Females were mated to a male of the same stock and proven fertility at a ratio of three females to one male. Each cage was divided into two parts by a guillotine door, separating the sexes until 3 a.m. on the mating day, when the door opened automatically. Successful mating was assessed by the presence of a vaginal plug or of spermatozoa in a vaginal smear. The day of successful mating was designated as gestation day (GD) 0.

3. Animal assignment and dose selection are presented in Table 1. Animals were assigned to a control or treatment group using a method of randomization based on weight stratification.

TABLE 1. Animal assignment

Test Group	Dose Level (mg/kg/day)	Number Assigned
Control	0	24
Low Dose	10	24
Mid Dose	100	24
High Dose	1000	24

Data taken from text tables pp. 16 and 17, MRID 44929510.

4. Dose selection rationale

Doses were selected on the basis of a range-finding study (Laboratory No. 911361) in pregnant rats. In this study, no maternal or developmental toxicity was observed at doses of 500 or 1000 mg/kg/day. Further details of this study were not included in the report.

5. Dose solution preparation and analysis

The test substance was mixed in a 0.5% aqueous solution of sodium carboxymethyl-cellulose. Solutions were prepared daily with a high-speed homogenizer. Homogeneity during administration was maintained with a magnetic stirrer. Samples of the dosing solutions were analyzed for concentration, homogeneity, and stability twice during the study. Samples from the top, middle, and bottom of the dosing solutions were analyzed for concentration and homogeneity. Stability was determined after 2 hours at room temperature from samples taken from the middle of the solutions.

Results -

Concentration analysis: Absence of test article was confirmed in the vehicle. Concentrations of the dosing solutions ranged from 92.0% to 102.8% of nominal.

Homogeneity analysis: Concentrations of the top, middle, and bottom of the dosing solutions differed by <4%.

Stability analysis: After 2 hours, concentrations of the dosing solutions ranged from 94.4% to 105.9% of their initial measured concentrations.

Analyses of the dosing solutions indicated that the test article could be adequately mixed in the vehicle, was stable for the duration of use, and that actual doses to the animals were acceptable.

6. Dosing

All doses were administered in a volume of 10 mL/kg of body weight.

C. OBSERVATIONS

1. Maternal observations and evaluations

The animals were checked daily for clinical signs and mortality. Body weights were measured daily and food consumption was measured on days 6, 11, 16, and 21. Dams were sacrificed on GD 21 by carbon dioxide inhalation and examined grossly. The number of corpora lutea on each ovary was counted. Gravid uteri were weighed and examined for number and location of live and dead fetuses, early and late resorptions, and abortion sites. Uteri that appeared nongravid were placed in ammonium sulfide to visualize possible implantation sites. Dams found dead or sacrificed early were subjected to gross necropsy.

2. Fetal evaluations

At necropsy, each live fetus was weighed, sexed, and examined for external abnormalities. Fetuses were killed by subcutaneous injection of a barbiturate anesthetic. Approximately one-half of each litter was processed for visceral examination and the remaining one-half was processed for skeletal examination. In the case of a gross external anomaly or malformation, fetuses were allocated to one technique depending on the type and incidence of the finding. For the visceral examinations, fetuses were fixed in Bouin's solution for at least two weeks and then micro-dissected. For the skeletal examinations, fetuses were cleared with potassium hydroxide and stained with alizarin red S.

D. DATA ANALYSIS

1. Statistical analysis

Continuous data were analyzed by the Analysis of Variance (ANOVA) followed by Dunnett's t-test to separate the means. The Chi-Square and Fisher's Exact tests were used for the analysis of categorical data. Non-parametric data were analyzed with the Kruskal-Wallis test followed by the Mann-Whitney U test.

2. Historical control data from January 1, 1988 to March 1, 1992 on 624 mated females were provided to allow comparison with concurrent controls and treatment groups.

II. RESULTS

A. MATERNAL TOXICITY

1. Mortality and clinical signs

One low-dose dam was sacrificed on GD 20 following observations of weight loss and inflammation of the vulva. All remaining animals survived to scheduled sacrifice. Necropsy showed a congested, dilated bladder and a white-yellowish discharge indicative of a uro-genital infection. No treatment-related clinical signs of toxicity were observed in any animal.

2. Body weight

Selected maternal body weights during gestation are given in Table 2. No statistically significant differences in absolute body weights occurred at any time between the treated groups and the control group. Body weight gains were also similar between the treated and control groups throughout the study.

HI

TABLE 2: Maternal body weights during gestation (g)				
GD	**0 mg/kg/day**	**10 mg/kg/day**	**100 mg/kg/day**	**1000 mg/kg/day**
0	196.3 ± 10.6	195.8 ± 11.5	195.1 ± 10.5	196.1 ± 12.8
6	225.2 ± 13.6	226.3 ± 13.6	226.9 ± 11.9	227.4 ± 13.4
10	245.3 ± 13.2	246.7 ± 14.6	249.7 ± 14.0	246.1 ± 15.9
16	289.3 ± 15.0	295.4 ± 17.3	296.4 ± 18.0	288.0 ± 19.8
21	360.9 ± 25.1	372.8 ± 27.6	369.4 ± 33.0	364.6 ± 31.4
Adjusted body wt.[a]	263.2	265.5	274.1	263.1

Data taken from Tables 2 and 7, pp. 32-34 and 47, respectively, MRID 44929510.
[a]Adjusted body weight = terminal body weight - gravid uterine weight.

3. Food consumption

There were no dose- or treatment-related differences in food consumption between treated and control groups at any time during gestation. High-dose dams ate significantly (91.9% of control; $p \leq 0.05$) less food than the controls on GD 6-11, but no other differences were noted either during or after the treatment interval.

4. Gross pathology

No treatment-related gross abnormalities were observed at maternal necropsy. Evidence of a urogenital infection was seen in the low-dose dam sacrificed on GD 20.

5. Cesarean section data

Data collected at cesarean section are summarized in Table 3. No differences were observed between the treated and control groups for number of corpora lutea, number of implantation sites, live fetuses/dam, resorptions, pre- and post-implantation losses, fetal body weights, or fetal sex ratios. All pregnant dams had live fetuses at necropsy.

TABLE 3: Cesarean section observations				
Observation	**0 mg/kg/day**	**10 mg/kg/day**	**100 mg/kg/day**	**1000 mg/kg/day**
No. Animals Assigned	24	24	24	24
No. Animals Pregnant	21	22	22	23
Pregnancy Rate (%)[a]	87.5	91.7	91.7	95.8
Maternal Mortality	0	1	0	0
Delivered Early/Aborted	0	0	0	0
Gravid Uterine Wt (g)	97.6	107.3	95.3	101.4
Corpora Lutea/Dam	14.3	16.5	14.8	15.3
Implantation/Dam	13.3	15.2	13.6	14.0
Preimplantaion Loss (mean %)	7.5	8.0	9.0	10.2
Postimplantaion Loss (mean %)	3.9	4.7	8.7	2.9
Total Live Fetuses	268	321	279	312
Live Fetuses/Litter	12.8	14.6	12.7	13.6
Mean Fetal Weight (g)	5.5	5.4	5.3	5.6
Sex Ratio (% Male)	50.4	49.8	44.1	51.6
Total Dead Fetuses	0	1	0	0
Dams With All Resorptions	0	0	0	0
Resorptions/Dam				
Early Resorptions	0.6	0.5	1.0	0.4
Late Resorptions	0.0	0.0	0.0	0.0

Data taken from Tables 5, 6, and 7, pp. 41, 43-45, and 47, respectively, MRID 44929510.
[a]Calculated by reviewer.

B. DEVELOPMENTAL TOXICITY

No treatment-related external, visceral, or skeletal malformations/variations were observed in any fetus from any group. A summary of these findings is given in Table 4.

1. External examination

The number of fetuses(litters) examined for external malformations/variations in the 0, 10, 100, and 1000 mg/kg/day groups was 268(21), 321(22), 279(22), and 312(23), respectively. A protruding tongue was seen in one control fetus. One low-dose litter

contained a fetus with a position anomaly of the hindlimb and another fetus with generalized edema.

2. Visceral examination

The number of fetuses(litters) examined for visceral malformations/variations in the 0, 10, 100, and 1000 mg/kg/day groups was 129(21), 153(22), 135(22), and 150(23), respectively. Anomalies such as hypertrophy of the left heart ventricle, renal pelvic dilatation, blood stained fluid in the abdominal cavity, enlarged thymus, and accessory lobulets on the liver were seen in one to two fetuses per group including controls.

3. Skeletal examination

The number of fetuses(litters) examined for skeletal malformations/variations in the 0, 10, 100, and 1000 mg/kg/day groups was 139(21), 167(22), 144(22), and 162(23), respectively. Skeletal anomalies of the sternebrae, vertebrae, and ribs were observed at low incidences in fetuses from the treated and control groups. Variations in ossification rates of the cranial bones, metatarsals, sternebrae, calcaneus, vertebrae, ribs, and phalanges were also common to fetuses from all groups.

TABLE 4: Fetal external, visceral, and skeletal observations (no. fetuses [no. litters] affected)

Observation	0 mg/kg/day	10 mg/kg/day	100 mg/kg/day	1000 mg/kg/day
External				
Total external findings	1 (1)	2 (1)	0 (0)	0 (0)
Visceral				
Total visceral findings	4 (3)	1 (1)	2 (2)	2 (2)
Enlarged thymus	1 (1)	0 (0)	1 (1)	1 (1)
Hypertrophy left ventricle	1 (1)	0 (0)	0 (0)	0 (0)
Blood stained fluid in abdominal cavity	0 (0)	0 (0)	0 (0)	1 (1)
Renal pelvic dilatation	0 (0)	1 (1)	0 (0)	0 (0)
Accessory liver lobulet	2 (2)	0 (0)	1 (1)	0 (0)
Skeletal				
Total skeletal malformations	0 (0)	0 (0)	0 (0)	0 (0)
Total skeletal anomalies	5 (5)	2 (2)	7 (7)	7 (6)
Total skeletal variations	139 (21)	167 (22)	144 (22)	162 (23)

Data taken from Tables 9, 10, and 14, pp. 51-52, 54-55, and 78, respectively, MRID 44931710.

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III. DISCUSSION

A. INVESTIGATORS' CONCLUSIONS

The study author concluded that CGA 51202 Technical resulted in maternal toxicity as evidenced by reduced food consumption in the 1000 mg/kg/day group on GD 6-11. The maternal toxicity NOEL was 100 mg/kg/day.

No test article related effects in the reproductive parameters were noted. No evidence of a "teratogenic potential" was apparent. Therefore, the developmental toxicity NOEL was 1000 mg/kg/day.

B. REVIEWER'S DISCUSSION

1. MATERNAL TOXICITY

Maternal toxicity was not evident in any treated group. No clinical signs were observed and body weights and body weight gains were similar between the treated and control groups. The lower food consumption by the high-dose group during GD 6-11 was within 9% of the control level, was not accompanied by reductions in body weight gains by these dams, was not dose-related, and is not considered to be biologically significant. Therefore, the transient reduction in food consumption by the high-dose dams is not considered by the reviewer to be an adverse affect of treatment.

Therefore, the maternal toxicity NOAEL is ≥1000 mg/kg/day and the maternal toxicity LOAEL was not identified.

2. DEVELOPMENTAL TOXICITY

a. Deaths/resorptions

Maternal treatment with the test article did not result in increases in either pre- or postimplantation loss or fetal death.

b. Altered growth

No treatment-related effects on fetal body weights or ossification rates were observed.

c. Developmental variations

Developmental variations were common to both treated and control fetuses and the incidence rates of specific variations were not affected by treatment.

d. Malformations

Malformations did not increase with exposure to the test article. The only major malformation described was generalized edema in one low-dose fetus.

Therefore, the developmental toxicity NOAEL is ≥1000 mg/kg/day and the developmental toxicity LOAEL was not identified.

It should be noted that although neither maternal nor developmental toxicity were apparent, the high dose is equivalent to the limit dose for developmental toxicity studies.

C. STUDY DEFICIENCIES

No deficiencies were identified that would compromise the integrity of this study.

D. CORE CLASSIFICATION

This study is classified as **Acceptable/Guideline** and satisfies the requirements for a developmental toxicity study [870.3700 (§83-3a)] in rats.

DATA EVALUATION REPORT

CGA-51202

STUDY TYPE: *IN VIVO* MAMMALIAN CYTOGENETICS - MICRONUCLEUS ASSAY IN MOUSE BONE MARROW CELLS [OPPTS. 870.5395(§84-2)]
MRID 44929511

Prepared for

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Toxicology and Risk Analysis Section
Life Sciences Division
Oak Ridge National Laboratory*
Oak Ridge, TN 37831
Task Order No. 00-10H

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Disclaimer

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

Oak Ridge National Laboratory, managed by Lockheed Martin Energy Research Corp. for the U.S. Department of Energy under contract number DE-AC05-96OR22464.

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Reregistration Branch I, Health Effects Division (7509C)
EPA Work Assignment Manager: Joycelyn Stewart, PhD Joycelyn E Stewart, Date 4/9/01
Toxicology Branch, Health Effects Division (7509C)

DATA EVALUATION RECORD

STUDY TYPE: *In vivo* mammalian cytogenetics - micronucleus assay in mouse bone marrow cells [OPPTS 870.5395 (§84-2)]

DP BARCODE: D260000 SUBMISSION CODE: S569354
P.C. CODE: 108801 (parent) TOX. CHEM. NO.:188DD

TEST MATERIAL (PURITY): CGA-51202 technical (CGA-51202, 100% a.i.)

SYNONYMS: Metochlor OA

CITATION: Hertner, Th. (1992) CGA-51202: Final Report - Micronucleus test, mouse. CIBA-GEIGY Limited, Genetic Toxicology, Basle, Switzerland. Laboratory study ID 911343, Novartis No. 410-91, August 28, 1992. MRID 44929511. Unpublished.

SPONSOR: Novartis Crop Protection, Inc., 410 Swing Road, Post Office Box 18300, Greensboro, NC 27419

EXECUTIVE SUMMARY: In a Tif: MAGf (SPF) mouse bone marrow micronucleus assay (MRID 44929511), five mice/sex/dose were treated once via oral gavage with CGA-51202 technical (Batch No. JD 7069/3, 100% a.i.) at doses of 600, 1200 and 2400 mg/kg body weight. In an initial micronucleus assay, bone marrow cells were harvested at 16, 24 and 48 hours post-treatment from test material treated mice and at 24 hours post-treatment from solvent and positive control treated mice. In a second assay, harvest times were 24 and 48 hours post-treatment for the high dose and solvent control mice and 24 hours post-treatment for the intermediate and low dose mice and for the positive control. The vehicle was Arachis oil.

There were signs of toxicity during the study. A preliminary toxicity test was conducted with concentrations up to 3000 mg/kg, the solubility limit using one male and one female per dose. Both animals in each group survived the treatments (three day observation period) but both mice receiving 3000 mg/kg showed respiratory sounds. A confirmatory assay was conducted with 3000 mg/kg using one male and one female. Respiratory sounds were heard in both mice and the male mouse died two days after treatment. An upper dose of 2400 mg/kg was thus selected for the micronucleus assay. In the initial micronucleus assay, data from the 24 and 48 hour harvest times were not acceptable because the analytically determined test material concentrations were too low (the results were not shown). Clinical signs of toxicity seen in mice from the 2400 mg/kg 16 hour sampling time group were respiratory sounds, piloerection and dyspnea. The PCE/NCE ratios did not indicate bone marrow cytotoxicity at any test material dose. No statistically significant increase in the number of micronucleated PCEs over the solvent control

value was seen at any CGA-51202 technical concentration at the 16 hour sampling time. The mean percentage of micronucleated PCEs (pooled male and female data in the absence of sex differences) was 0.04%, 0.05% and 0.05% in the 600, 1200 and 2400 mg/kg treatment groups, respectively, compared to the solvent control value of 0.02%. The positive control value was 1.15%, significant at $p<0.05$. A second micronucleus assay was conducted with doses of 600, 1200 and 2400 mg/kg and a 24 hour harvest time. An additional harvest time of 48 hours was used with the 2400 mg/kg group. At the 24 hour harvest time, three females and two males from the 2400 mg/kg groups had respiratory sounds while all other mice in the upper dose groups and all mice in the lower two dose groups appeared normal. In the 48 hour harvest time groups, all animals except one male had one or more of the following signs of toxicity: respiratory sounds, reduced locomotor activity, piloerection, tremor and dyspnea. The PCE/NCE ratios did not indicate bone marrow cytotoxicity at any test material dose. A statistically significant increase ($p<0.05$) in the mean number of micronucleated PCEs (pooled male and female data) over the solvent control value (0.01%) was seen at 600 (0.08%) and 1200 (0.08%) but not at 2400 mg/kg (0.04%). Although statistically significant due to the very low solvent control value, the actual percentages of micronucleated PCEs were well below the laboratory's criterion for a positive response of 0.20%, thus the results were not considered biologically significant. The positive control value of 0.98% was appropriate. The mean percentage of micronucleated PCEs at the 48 hour harvest time (2400 mg/kg) was 0.05% compared to 0.10% for the solvent control. **There was no biologically significant increase in the frequency of micronucleated polychromatic erythrocytes in bone marrow after any dose or treatment time used in the study.**

This study is classified as **Acceptable/Guideline**. It satisfies the requirement for FIFRA Test Guideline [OPPTS 870.5395 (§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: CGA-51202 technical

Description: beige crystalline material
Lot/Batch #: JD 7069/3
Purity: 100% a.i.
Stability of compound: responsibility of sponsor
CAS #: not provided
Structure: not provided
Solvent used: Arachis oil
Other comments: none

2. Control materials

Negative *(if not vehicle)*/Route of administration: none

Vehicle/Final volume/Route of administration: Arachis oil / 10 mL/kg / oral gavage

Positive/Final dose/Route of administration: Cyclophosphamide / 64 mg/kg / oral gavage

3. Test compound administration

Volume of test substance administered: 10 mL/kg body weight
Route of administration: oral gavage
Dose levels used:
Preliminary toxicity test: 187.5, 750, 3000 mg/kg
Micronucleus test: 600, 1200, 2400 mg/kg

4. Test animals

Species: mouse
Strain: Tif: MAGf (SPF)
Age: "young"
Weight (micronucleus test): male 29-39 g female 26-36 g
Weight (toxicity test) male: 23-27 g female 25-28 g
Source: CIBA-GEIGY Animal Farm, Sisseln, Switzerland
No. animals used per dose: 5 males 5 females
Properly maintained? Y

B. TEST PERFORMANCE

1. Treatment and sampling times

a.. Test compound

Dosing: _x_ once ___ twice (24 hr apart)
Sampling (after last dose): ___ 6 hr ___ 12 hr _x_ 24 hr _x_ 48 hr ___ 72 hr,
_x_ other _x_ 16 hr

b. Negative and/or vehicle control

Dosing: _x_ once ___ twice (24 hr apart)
Sampling (after last dose): ___ 6 hr ___ 12 hr
_x_ 24 hr _x (second assay only)_ 48 hr ___ 72 hr, other

c. Positive control

Dosing: _x_ once ___ twice (24 hr apart)
Sampling (after last dose): ___ 6 hr ___ 12 hr
_x_ 24 hr ___ 48 hr ___ 72 hr

2. Tissues and cells examined

_x_ bone marrow ___ other:
No. of polychromatic erythrocytes (PCE) examined per animal: _1000_
No. of normochromatic erythrocytes (NCE; more mature RBCs) examined per animal: _the number found while scoring 1000 PCEs_

3. Details of slide preparation

Mice were killed by cervical dislocation and the bone marrow harvested from both femurs of each mouse using fetal calf serum. Nucleated cells were removed using a cellulose column. A 10 µm filter was attached to a syringe filled with 0.3-1.0 g of a mixture (1:1 w/w) of microcrystalline cellulose (Sigmacell type 50) and α-cellulose fibers. The bone marrow suspension was placed on top of the column and eluted with Hank's BSS buffer. The eluate containing the erythrocytes was centrifuged and the cells resuspended in fetal calf serum. A sample of the cell suspension was placed on a slide, smeared, air-dried and stained with May-Grünwald/Giemsa solution. The slides were rinsed with distilled water, air-dried, cleared in Xylene and mounted. Prior to scoring, the slides were coded.

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4. Statistical methods

The significance of differences was assessed by the Chi-Square test ($p<0.05$).

5. Evaluation criteria

Micronuclei were identified as uniform, darkly stained, roundish bodies in the cytoplasm. One-thousand PCE per mouse were scored and the results reported as the number of micronucleated PCEs/1000 PCEs. A micronucleated PCE could contain one or more micronuclei. The PCE/NCE ratio was also recorded.

Results were considered positive if the mean number of micronucleated PCEs in a test material treated group was greater than 0.20% and if there was a statistically significant (Chi Squared < 3.84) increase in the mean number of micronucleated PCEs in the treated group compared to the solvent control. If the positive result was seen in a minority of mice in a group and if the number of micronucleated NCEs was also increased, the effects were considered unrelated to the test material.

II. REPORTED RESULTS

A. PRELIMINARY TOXICITY ASSAY

Three doses of CGA-51202 technical (187.5, 750 and 3000 mg/kg) were tested in an initial toxicity assay using one male and one female for each dose. The upper dose was limited by solubility. All animals survived the treatments (three day observation period) but both mice receiving 3000 mg/kg showed respiratory sounds. A confirmatory assay was conducted with 3000 mg/kg using one male and one female. Respiratory sounds were heard in both mice and the male mouse died two days after treatment. An upper dose of 2400 mg/kg was selected for the micronucleus assay.

B. MICRONUCLEUS ASSAY

Three doses of CGA-51202 technical (600, 1200 and 2400 mg/kg) were tested in the initial micronucleus assay using five mice/sex/dose. Bone marrow cells were harvested at 16, 24 and 48 hours post-treatment. Data from the 24 and 48 hour harvest times were not acceptable because the analytically determined test material concentrations were too low (the results not shown). Clinical signs of toxicity seen in mice from the 2400 mg/kg 16 hour sampling time group were respiratory sounds, piloerection and dyspnea. The PCE/NCE ratios did not indicate bone marrow cytotoxicity at any test material dose. No statistically significant increase in the number of micronucleated PCEs over the solvent control value was seen at any CGA-51202 technical concentration at the 16 hour sampling time. The mean percentage of micronucleated PCEs (pooled male and female data in the absence of sex differences) was 0.04%, 0.05% and 0.05% in the 600, 1200 and 2400 mg/kg treatment groups, respectively, compared to the solvent control value of 0.02%. The positive control value was 1.15%, significant at $p<0.05$. Results of the initial

micronucleus assay are summarized in Appendix Tables 1 and 2 (MRID 44929511, pp. 27 and 28).

A second micronucleus assay was conducted with doses of 600, 1200 and 2400 mg/kg and a 24 hour harvest time. An additional harvest time of 48 hours was used with the 2400 mg/kg group. At the 24 hour harvest time, three females and two males from the 2400 mg/kg groups had respiratory sounds while all other mice in the upper dose groups and all mice in the lower two dose groups appeared normal. In the 48 hour harvest time groups, all animals except one male had one or more of the following signs of toxicity: respiratory sounds, reduced locomotor activity, piloerection, tremor and dyspnea. The PCE/NCE ratios did not indicate bone marrow cytotoxicity at any test material dose. A statistically significant increase ($p<0.05$) in the mean number of micronucleated PCEs (pooled male and female data) over the solvent control value (0.01%) was seen at 600 (0.08%) and 1200 (0.08%) but not at 2400 mg/kg (0.04%). Although statistically significant due to the very low solvent control value, the actual percentages of micronucleated PCEs were well below the laboratory's criterion for a positive response of 0.20%, thus the results were not considered biologically significant. The positive control value of 0.98% was appropriate. The mean percentage of micronucleated PCEs at the 48 hour harvest time (2400 mg/kg) was 0.05% compared to 0.10% for the solvent control. Results of the second micronucleus are summarized in Appendix Tables 3 and 4 (MRID 44929511, pp. 29 and 30).

CGA-51202 technical did not increase the number of micronucleated PCEs over solvent control values as tested in this study.

III. REVIEWER'S DISCUSSION/CONCLUSIONS

A. This is an acceptable study. CGA-51202 technical was tested to a dose limited by solubility and toxicity, proper experimental protocol was followed and the solvent and positive control values were appropriate. The reviewers agree with the author's conclusion that the statistically significant increases in the number of micronucleated PCEs seen in the second experiment at the two lower doses were not biologically significant. As tested in this study, CGA-51202 technical did not increase the incidence of micronucleated bone marrow PCEs.

This study is classified as **Acceptable/Guideline**. It satisfies the requirement for FIFRA Test Guideline [OPPTS 870.5395 (§84-2)] for *in vivo* cytogenetic mutagenicity data.

B. STUDY DEFICIENCIES

No study deficiencies were identified.

APPENDIX

MRID 44929511

THE FOLLOWING ATTACHMENTS ARE NOT AVAILABLE ELECTRONICALLY. SEE THE FILE COPY.

TABLE 1 : MICRONUCLEUS TEST ON MOUSE BONE MARROW CELLS
SUMMARIZED DATA (first experiment)

ANIMALS SACRIFICED 16 h AFTER APPLICATION

Test number : 911343
Test substance : CGA 51202 tech.
Batch : JD 7069/3

Treatment Sex	PCEs per 1000 erythrocytes (mean)	Ratio PCE/ NCE	Micronucleated PCEs found in 5000 PCEs #	% of micro-nucleated PCEs
600.0 mg/kg				
Males	437	0.78	2	0.04
Females	506	1.02	2	0.04
Pooled data			4	0.04
1200.0 mg/kg				
Males	417	0.72	3	0.06
Females	475	0.91	2	0.04
Pooled data			5	0.05
2400.0 mg/kg				
Males	389	0.64	2	0.04
Females	469	0.88	3	0.06
Pooled data			5	0.05

In a total of 5 animals

TABLE 2 : MICRONUCLEUS TEST ON MOUSE BONE MARROW CELLS
SUMMARIZED DATA (first experiment)

ANIMALS SACRIFICED 24 h AFTER APPLICATION

Test number : 911343
Test substance : CGA 51202 tech.
Batch : JD 7069/3

Treatment Sex	PCEs per 1000 erythrocytes (mean)	Ratio PCE/ NCE	Micronucleated PCEs found in 5000 PCEs #	% of micronucleated PCEs
Negative Control: Arachis Oil				
Males	359	0.56	0	0.00
Females	434	0.77	2	0.04
Pooled data			2	0.02
Positive Control: Cyclophosphamide, 64 mg/kg				
Males	387	0.63	63	1.26*
Females	411	0.70	52	1.04*
Pooled data			115	1.15*

In a total of 5 animals
* Significantly different from neg. contr. at a level of significance of p = 0.05

TABLE 3 : MICRONUCLEUS TEST ON MOUSE BONE MARROW CELLS
SUMMARIZED DATA (second experiment)

ANIMALS SACRIFICED 24 h AFTER APPLICATION

Test number : 911343
Test substance : CGA 51202 tech.
Batch : JD 7069/3

Treatment Sex	PCEs per 1000 erythrocytes (mean)	Ratio PCE/ NCE	Micronucleated PCEs found in 5000 PCEs #	% of micro-nucleated PCEs
Negative Control: Arachis Oil				
Males	446	0.81	1	0.02
Females	388	0.63	0 !	0.00
Pooled data			1	0.01
600.0 mg/kg				
Males	433	0.76	4	0.08
Females	485	0.94	4	0.08
Pooled data			8	0.08*
1200.0 mg/kg				
Males	330	0.49	6	0.12
Females	489	0.96	2	0.04
Pooled data			8	0.08*
2400.0 mg/kg				
Males	547	1.21	1	0.02
Females	497	0.99	3	0.06
Pooled data			4	0.04
Positive Control: Cyclophosphamide, 64 mg/kg				
Males	436	0.77	67	1.34*
Females	326	0.48	31	0.62*
Pooled data			98	0.98*

In a total of 5 animals
! Only 4 animals used
* Significantly different from neg. contr. at a level of significance of p = 0.05

TABLE 4 : MICRONUCLEUS TEST ON MOUSE BONE MARROW CELLS
SUMMARIZED DATA (second experiment)

ANIMALS SACRIFICED 48 h AFTER APPLICATION

Test number : 911343
Test substance : CGA 51202 tech.
Batch : JD 7069/3

Treatment Sex	PCEs per 1000 erythrocytes (mean)	Ratio PCE/ NCE	Micronucleated PCEs found in 5000 PCEs #	% of micro-nucleated PCEs
Negative Control: Arachis Oil				
Males	473	0.90	5	0.10
Females	510	1.04	5	0.10
Pooled data			10	0.10
2400.0 mg/kg				
Males	410	0.69	4	0.08
Females	529	1.12	1	0.02
Pooled data			5	0.05

In a total of 5 animals

DATA EVALUATION REPORT

CGA-51202

***SALMONELLA/ESCHERICHIA*/MAMMALIAN ACTIVATION GENE MUTATION ASSAY; [OPPTS 870.5100 (§84-2)]**
MRID 44929512

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
Life Sciences Division
Oak Ridge National Laboratory
Oak Ridge, TN 37831
Task Order No. 00-10I

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Disclaimer

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

Managed by Lockheed Martin Energy Research Corp. for the U.S. Department of Energy under Contract No. DE-AC05-96OR22464.

EPA Reviewer: Virginia A. Dobozy, VMD, MPH Virginia A Dobozy, Date 7/26/01
Reregistration Branch I, Health Effects Division (7509C)
EPA Work Assignment Manager: Joycelyn Stewart, PhD Joycelyn Stewart, Date 7/31/2001
Toxicology Branch, Health Effects Division (7509C)

DATA EVALUATION RECORD

STUDY TYPE: *Salmonella/Escherichia*/mammalian activation gene mutation assay [OPPTS 870.5100 (§84-2)]

DP BARCODE: D260000
P.C. CODE: 108801 (parent)

SUBMISSION CODE: S569354
TOX. CHEM. NO.:188DD

TEST MATERIAL (PURITY): CGA-51202 technical (100% a.i.)

SYNONYMS: none provided

CITATION: Hertner, Th. (1992) *Salmonella* and *Escherichia*/liver-microsome test. CIBA-GEIGY Limited, Genetic Toxicology, Basle, Switzerland. Laboratory study ID 911342, Novartis No. 411-91, March 20, 1992. MRID 44929512. Unpublished.

SPONSOR: Novartis Crop Protection, Inc., 410 Swing Road, Post Office Box 18300, Greensboro, NC 27419

EXECUTIVE SUMMARY In a reverse gene mutation assay in bacteria (MRID 44929512), strains TA98, TA100, TA1535 and TA1537 of *S. typhimurium* and strain WP2(uvrA) of *E. coli* were exposed to CGA-51202 technical (Batch No. JD 7069/3, 100% a.i.) in DMSO at concentrations of 312.5, 625.0, 1250.0, 2500.0, 5000.0 µg/plate in the presence and absence of mammalian metabolic activation. Two independent assays were conducted and all plating was in triplicate. The S9-fraction was obtained from Aroclor induced male RAI (Tif:RAIf(SPF)) rat liver.

CGA-51202 technical was tested up to a limit concentration of 5000 µg/plate. Cytotoxicity, as based on a reduction in the number of revertants per plate compared to the solvent control value, was seen in a preliminary cytotoxicity assay using TA100 and WP2(uvrA) only at 5000 µg/plate without S9-mix. The number of revertants per plate was 54% of the solvent control value in TA100 and 53% of the solvent control value in WP2(uvrA). A precipitate was seen on the TA100 plate at 5000 µg/plate without S9-mix. In the mutagenicity assays, no cytotoxicity was evident at any test point and no precipitates were seen. CGA-51202 technical did not increase the number of revertants per plate over solvent control values at any tested concentration in any of the five bacterial strains in either mutagenicity assay, with or without S9-mix. The solvent and positive control values were appropriate for the respective strains and within the laboratory's historical control ranges. **There was no evidence of induced mutant colonies over background.**

This study is classified as **Acceptable/Guideline**. It satisfies the requirement for FIFRA Test Guideline 84-2 (OPPTS 870.5100) 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: CGA-51202 technical

Description: beige crystalline material
Lot/Batch #: JD 7069/3
Purity: 100% a.i.
Stability of compound: responsibility of sponsor
CAS #: not provided
Structure: not provided
Solvent used: DMSO
Other comments: none

2. Control materials

Negative: none
Solvent/final concentration: DMSO

Positive:
Nonactivation:
Sodium azide 5.0 µg/plate TA100, TA1535
2-Nitrofluorene 20.0 µg/plate TA98
9-Aminoacridine 150.0 µg/plate TA1537

Other:
4-nitroquinoline-N-oxide 2.0 µg/plate WP2(uvrA)

Activation:
2-Aminoanthracene 2.5 µg/plate TA98, TA100, TA1537
2-Aminoanthracene 50.0 µg/plate WP2(uvrA)
Cyclophosphamide 400.0 µg/plate TA1535

3. Activation: S9 derived from male RAI (Tif:RAIf(SPF)) rats

x Aroclor 1254	x induced	x rat	x liver
__ phenobarbital	__ non-induced	__ mouse	__ lung
__ none		__ other	__ other

S9 mix composition (if purchased, give details):

S9-fraction	100 µL/mL
NADP	4 µmol/mL
$MgCl_2$	8 µmol/mL

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KCl	33 µmol/mL
Na-phosphate buffer, pH 7.4	100 µmol/mL
Glucose-6-phosphate	5 µmol/mL

4. Test organisms: *S. typhimurium* strains

__ TA97 _x_ TA98 _x_ TA100 __ TA102 __ TA104
_x_ TA1535 _x_ TA1537 ___ TA1538; list any others:

E. coli strain: WP2(uvrA)

Properly maintained? Y
Checked for appropriate genetic markers (rfa mutation, R factor)? Y

5. Test compound concentrations used:

Preliminary cytotoxicity assay: (TA100 and WP2(uvrA), single plating)
Nonactivated and activated conditions: 20.5761, 61.7284, 185.1852, 555.5556, 1666.6667, 5000.0000 µg/plate

Initial and confirmatory mutagenicity assay: (all strains, triplicate plating)

Nonactivated and activated conditions: 312.5, 625.0, 1250.0, 2500.0, 5000.0 µg/plate

B. TEST PERFORMANCE

1. Type of Salmonella assay:

_x_ standard plate test
__ pre-incubation
__ "Prival" modification *(i.e. azo-reduction method)*
__ spot test

2. Protocol

One-tenth mL of an overnight culture of bacteria was mixed with 2 mL of molten top agar, 0.1 mL of the desired concentration of test material, solvent or positive control and 0.5 mL of either sodium phosphate buffer or S9-mix. This mixture was poured into a Petri dish containing about 20 mL of minimal agar (1.5% agar supplemented with 2% salts of Vogel-Bonner Medium E and 2% glucose). The top agar (0.6% agar and 0.6% NaCl) was supplemented with 10% of 0.5 mM L-histidine and 0.5 mM (+)biotin dissolved in water for the *S. typhimurium* strains and with 10% of 0.5 mM L-tryptophan dissolved in water for the *E. coli* strain. The plates were inverted and incubated for 48 hours at 37 ± 1.5°C in the dark. The background lawn of bacteria was then examined and the number of revertant colonies counted with an Artek colony counter.

Criteria for a positive response are a reproducible doubling, at least, of the mean number of revertants per plate above that of the solvent control at any concentration for one or more of the following strains: TA98, TA1535, TA1537 and WP2(uvrA) or a 1.5 fold increase for TA100 accompanied by a positive dose-response.

II. REPORTED RESULTS

The concentrations of test material used in the assays were analytically determined (HPLC with UV detection) to be as intended.

A. PRELIMINARY CYTOTOXICITY ASSAY

Six concentrations of CGA-51202 technical ranging from 20.6 to 5000 µg/plate were tested with strains TA100 and WP2(uvrA) in the presence and absence of S9-mix. A single plate per dose/activation condition was used. The measure of cytotoxicity was a reduction in the number of revertants per plate in CGA-51202 treated cells compared to the solvent control value. No cytotoxicity was seen at any concentration in either strain in the presence of S9-mix or at the lower five concentrations in the absence of S9-mix. The number of revertants per plate at 5000 µg/plate without S9-mix was 54% of the solvent control value in TA100 and 53% of the solvent control value in WP2(uvrA). A precipitate was seen on the TA100 plate at 5000 µg/plate without S9-mix. The upper dose selected for the mutagenicity assays was 5000 µg/plate.

B. MUTAGENICITY ASSAY

Five concentrations of CGA-51202 technical ranging from 312.5 to 5000.0 µg/plate were tested, with and without S9-mix, in an initial and a confirmatory mutagenicity assay. All plating was in triplicate. No cytotoxicity as measured by a reduction in the number of revertants per plate compared to solvent control values was evident at any test point. No information was provided on the background lawn of bacteria. CGA-51202 technical did not increase the number of revertants per plate over solvent control levels at any tested concentration in any of the five bacterial strains in either mutagenicity assay, with or without S9-mix. The solvent and positive control values were appropriate for the respective strains and within the laboratory's historical control ranges (historical control data are included in the Appendix). Results of the initial mutagenicity assay are summarized in Appendix Tables 1 and 2 (MRID 44929512, pp. 30 and 31) and those of the confirmatory assay are summarized in Appendix Tables 3 and 4 (MRID 44929512, pp. 32 and 33).

III. REVIEWER'S DISCUSSION/CONCLUSIONS

A. This is an acceptable study. CGA-51202 technical was tested to a limit concentration of 5000 µg/plate, suitable experimental protocol was followed and the solvent and positive control values were appropriate for the respective strains. The test material did not increase the number of revertants per plate at any concentration as tested in this study.

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This study is classified as **Acceptable/guideline**. It satisfies the requirement for FIFRA Test Guideline 84-2 (OPPTS 870.5100) for *in vitro* mutagenicity [bacterial reverse gene mutation] data.

B. STUDY DEFICIENCIES

No study deficiencies were identified.

APPENDIX

MRID 44929512

THE FOLLOWING ATTACHMENTS ARE NOT AVAILABLE ELECTRONICALLY. SEE THE FILE COPY.

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Historical control data and acceptable ranges for controls

Arithmetic Mean (m) and Standard Deviation (s) of colony counts obtained in 91 separate experiments over the period of January 15, 1990 to January 02, 1991. Acceptable range for mean colony counts (=mean of values) of spontaneous revertants.

Without microsomal activation

Strain	Substance	µg/plate	m	s	Acceptable range
TA 100	negative control		146.9	28.2	80-220
	sodium azide	2.0	762.9	257.2	
TA 1535	negative control		11.4	3.3	7- 30
	sodium azide	2.0	699.4	277.8	
E.coli WP2 uvrA	negative control		20.2	4.5	8- 40
	4-nitroquinoline-N-oxide	1.0	501.7	323.4	
TA 98	negative control		19.1	4.4	12- 50
	2-nitrofluorene	10.0	1287.5	308.9	
TA 1537	negative control		7.9	2.6	3- 20
	9-aminoacridine	150.0	2158.9	583.3	

With microsomal activation

Strain	Substance	µg/plate	m	s	Acceptable range
TA 100	negative control		142.2	25.8	70-220
	2-aminoanthracene	2.5	1768.8	544.2	
TA 1535	negative control		12.6	3.2	7- 35
	cyclophosphamide	400.0	349.6	126.1	
E.coli WP2 uvrA	negative control		22.3	5.2	8- 50
	2-aminoanthracene	50.0	1109.7	277.1	
TA 98	negative control		35.4	5.7	20- 70
	2-aminoanthracene	2.5	1759.9	470.3	
TA 1537	negative control		12.0	4.3	5- 30
	2-aminoanthracene	5.0	204.3	82.4	

TABLE 1

SUMMARY OF THE RESULTS

Experiment without microsomal activation

Test number : 911342 Experiment : Original
Test substance : CGA 51202 tech. Batch : JD 7069/3

Treatment/Strain	TA 100	TA 1535	WP2 uvrA	TA 98
Negative control	151.3	8.3	19.3	23.0
CGA 51202 tech.:				
312.5000 ug/plate	144.3	7.7	14.3	25.7
625.0000 ug/plate	138.7	9.0	23.3	17.0
1250.0000 ug/plate	142.3	8.7	16.3	21.3
2500.0000 ug/plate	140.3	12.0	19.3	17.3
5000.0000 ug/plate	151.3	7.7	18.0	18.0
Positive controls:				
sodium azide	1472.3	1124.7	----	----
4-NQO	----	----	1020.7	----
2-nitrofluorene	----	----	----	2129.0

Treatment/Strain	TA 1537
Negative control	13.0
CGA 51202 tech.:	
312.5000 ug/plate	11.0
625.0000 ug/plate	13.0
1250.0000 ug/plate	12.3
2500.0000 ug/plate	13.7
5000.0000 ug/plate	6.0
Positive controls:	
9-aminoacridine	1658.3

TABLE 2

SUMMARY OF THE RESULTS

Experiment with microsomal activation

Test number : 911342 Experiment : Original
Test substance : CGA 51202 tech. Batch : JD 7069/3

Treatment/Strain	TA 100	TA 1535	WP2 uvrA	TA 98
Negative control	144.0	14.3	24.7	41.3
CGA 51202 tech.:				
312.5000 ug/plate	136.7	11.7	22.0	36.3
625.0000 ug/plate	157.7	6.0	24.3	37.0
1250.0000 ug/plate	134.7	8.7	20.0	33.0
2500.0000 ug/plate	138.3	8.3	19.0	43.3
5000.0000 ug/plate	128.0	10.7	15.0	37.0
Positive controls:				
2-aminoanthracene	2160.3	----	1037.0	1718.7
cyclophosphamide	----	635.0	----	----

Treatment/Strain	TA 1537
Negative control	11.7
CGA 51202 tech.:	
312.5000 ug/plate	12.3
625.0000 ug/plate	15.0
1250.0000 ug/plate	14.0
2500.0000 ug/plate	10.0
5000.0000 ug/plate	11.3
Positive controls:	
2-aminoanthracene	123.7

TABLE 3

SUMMARY OF THE RESULTS

Experiment without microsomal activation

Test number : 911342 Experiment : Confirmatory
Test substance : CGA 51202 tech. Batch : JD 7069/3

Treatment/Strain	TA 100	TA 1535	WP2 uvrA	TA 98
Negative control	102.7	11.0	21.0	19.0
CGA 51202 tech.:				
312.5000 ug/plate	109.7	8.7	24.3	18.7
625.0000 ug/plate	102.0	11.7	21.3	21.0
1250.0000 ug/plate	99.3	7.3	21.3	14.7
2500.0000 ug/plate	93.7	12.0	24.0	18.7
5000.0000 ug/plate	99.0	12.0	20.3	24.3
Positive controls:				
sodium azide	607.0	823.0	----	----
4-NQO	----	----	1164.7	----
2-nitrofluorene	----	----	----	1357.3

Treatment/Strain	TA 1537
Negative control	9.7
CGA 51202 tech.:	
312.5000 ug/plate	5.0
625.0000 ug/plate	10.7
1250.0000 ug/plate	9.3
2500.0000 ug/plate	6.0
5000.0000 ug/plate	5.7
Positive controls:	
9-aminoacridine	874.3

TABLE 4

SUMMARY OF THE RESULTS

Experiment with microsomal activation

Test number : 911342 Experiment : Confirmatory
Test substance : CGA 51202 tech. Batch : JD 7069/3

Treatment/Strain	TA 100	TA 1535	WP2 uvrA	TA 98
Negative control	103.0	9.7	17.0	32.3
CGA 51202 tech.:				
312.5000 ug/plate	102.3	11.7	20.0	39.0
625.0000 ug/plate	100.0	15.3	16.7	42.3
1250.0000 ug/plate	104.3	10.0	20.0	37.0
2500.0000 ug/plate	103.3	10.0	18.7	39.3
5000.0000 ug/plate	106.0	9.7	17.3	42.3
Positive controls:				
2-aminoanthracene	1349.3	----	1028.7	2414.7
cyclophosphamide	----	333.3	----	----

Treatment/Strain	TA 1537
Negative control	8.3
CGA 51202 tech.:	
312.5000 ug/plate	10.0
625.0000 ug/plate	8.0
1250.0000 ug/plate	12.7
2500.0000 ug/plate	12.0
5000.0000 ug/plate	6.0
Positive controls:	
2-aminoanthracene	100.7

DATA EVALUATION REPORT

METOLACHLOR ESA
(CGA-354743 TECHNICAL)

STUDY TYPE: ACUTE ORAL TOXICITY - RAT [870.1100 (§81-1)]
MRID 44931704

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. 00-09A

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Date: JAN 18 2000

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Signature: Robert H. Ross
Date: JAN 18 2000

Quality Assurance:
Lee Ann Wilson, M.A.

Signature: L. A. Wilson
Date: JAN 18 2000

Disclaimer

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

Oak Ridge National Laboratory, managed by Lockheed Martin Energy Research Corp. for the U.S. Department of Energy under contract number DE-AC05-96OR22464.

EPA Reviewer: Virginia A. Dobozy, VMD, MPH Virginia A Dobozy, Date 4/17/01
Reregistration Branch I, Health Effects Division (7509C)
EPA Work Assignment Manager: Joycelyn Stewart, PhD Joycelyn Stewart, Date 4/25/01
Toxicology Branch, Health Effects Division (7509C)

DATA EVALUATION RECORD

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

DP BARCODE: D260393 SUBMISSION CODE: S570059
P.C. CODE: 108801 (Metolachlor) TOX. CHEM. NO.: 188DD

TEST MATERIAL (PURITY): CGA-354743 Technical (metabolite of CGA 24705, 98.0% a.i.)

SYNONYMS: not reported

CITATION: Cantoreggi, S. (1998) CGA-354743: Acute oral toxicity in the rat (Limit test). Novartis Crop Protection AG, Toxicology, 4332 Stein, Switzerland. Laboratory study identification 981038, Novartis No. 647-98, June 19, 1998. MRID 44931704. Unpublished.

SPONSOR: Novartis Crop Protection, Inc., 410 Swing Road, P.O. Box 18300, Greensboro, NC 27419

EXECUTIVE SUMMARY: In an acute oral toxicity study (MRID 44931704) groups of five male and five female fasted young adult HanIbm:WIST rats were given a single oral 5000 mg/kg dose of CGA-354743 Technical (metabolite of CGA 24705, Batch No. KI-5408/6, 98.0% a.i.) in distilled water and observed for 14 days.

No rats died and all rats had normal body weight gains during the study. No remarkable clinical observations were noted during the study and no remarkable observations were noted at necropsy.

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

CGA-354743 Technical is in TOXICITY CATEGORY IV based on the LD_{50}.

This acute oral study is classified as **Acceptable/Guideline** and satisfies the 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: CGA-354743 Technical (metabolite of CGA 24705, metolachlor)

   Description: light brown solid
   Lot/Batch #: KI-5408/6
   Purity: 98.0% a.i.
   CAS #: not provided
   Structure: not provided

2. Vehicle and/or positive control

   Distilled water

3. Test animals

   Species: rat
   Strain: HanIbm:WIST
   Age and/or weight at dosing: young adult; males: 178-190 g, females: 170-180 g
   Source: BRL Biological Research Laboratories, Wölferstrasse 4, 4414 Füllinsdorf, Switzerland
   Acclimation period: at least 5 days
   Diet: NAFAG No. 890 (NAFAG, Gossau/SG, Switzerland), *ad libitum*
   Water: municipal water, *ad libitum*
   Housing: five same sex per Macrolon Type 4 cage
   Environmental conditions:
   Temperature: 21±3°C
   Humidity: 50±20%
   Air changes: approximately 13-14/hour
   Photoperiod: 12 hour light/12 hour dark

B. STUDY DESIGN AND METHODS

1. In life dates

   Start: April 28, 1998; end: May 12, 1998

2. Animal assignment and treatment

   Following an overnight fast, groups of five rats/sex were given by gavage a single 5000 mg/kg dose of the test material in distilled water. The animals were observed for clinical signs of toxicity at 1, 3, and 5 hours post dosing and daily thereafter for 14 days. Mortality checks were performed twice daily. They were weighed on study days 0, 7, and 14. All rats were sacrificed and necropsied.

TABLE 1. Doses, mortality/animals treated			
Dose (mg/kg)	**Males**	**Females**	**Combined**
5000	0/5	0/5	0/10

Data taken from Table 1, p. 14, MRID 44931704.

3. Statistics

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

II. RESULTS AND DISCUSSION

A. MORTALITY

Mortality is given in Table 1. None of the rats died as a result of CGA-354743 Technical toxicity.

The oral LD_{50} for males, females, and combined was > 5000 mg/kg. This places CGA-354743 Technical (metabolite of CGA 24705, metolachlor) in TOXICITY CATEGORY IV.

B. CLINICAL OBSERVATIONS

No remarkable clinical observations were noted.

C. BODY WEIGHT

All rats had normal body weight gains.

D. NECROPSY

No remarkable observations were noted.

E. DEFICIENCIES

None

DATA EVALUATION REPORT

METOLACHLOR ESA
(CGA-354743 TECHNICAL)

STUDY TYPE: ACUTE DERMAL TOXICITY - RAT [870.1200 (81-2)]
MRID 44931705

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. 00-09B

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Date: JAN 18 2000

Disclaimer

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Oak Ridge National Laboratory, managed by Lockheed Martin Energy Research Corp. for the U.S. Department of Energy under contract number DE-AC05-96OR22464.

EPA Reviewer: Virginia A. Dobozy, VMD, MPH Virginia A Dobozy, Date 4/17/01
Reregistration Branch I, Health Effects Division (7509C)
EPA Work Assignment Manager: Joycelyn Stewart, PhD Joycelyn Stewart, Date 4/20/01
Toxicology Branch, Health Effects Division (7509C)

DATA EVALUATION RECORD

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

DP BARCODE: D260393 SUBMISSION CODE: S570059
P.C. CODE: 108801 (Metolachlor) TOX. CHEM. NO.: 188DD

TEST MATERIAL (PURITY): CGA-354743 Technical (metabolite of CGA 24705, 98.0% a.i.)

SYNONYMS: not reported

CITATION: Cantoreggi, S. (1998) CGA-354743: Acute dermal toxicity in the rat (Limit test). Novartis Crop Protection AG, Toxicology, 4332 Stein, Switzerland. Laboratory study identification 981039, Novartis No. 645-98, June 19, 1998. MRID 44931705. Unpublished.

SPONSOR: Novartis Crop Protection, Inc., 410 Swing Road, P.O. Box 18300, Greensboro, NC 27419

EXECUTIVE SUMMARY: In an acute dermal toxicity study (MRID 44931705) approximately 10% of the body surface area of five male and five female young adult rats was dermally exposed to 2000 mg/kg (Limit Test) CGA-354743 Technical (metabolite of CGA 24705, Batch No. KI-5408/6, 98.0% a.i.) moistened with distilled water for 24 hours. The animals were observed for 14 days.

None of the animals died during the study. No remarkable clinical observations or local irritation were noted on any rats. With the exception of one female that lost weight during the first week, all animals had normal body weight gains. No observable abnormalities were noted at necropsy.

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

CGA-354743 Technical is in TOXICITY CATEGORY III based on the LD_{50}.

This acute dermal study is classified as **Acceptable/Guideline** and satisfies the 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: CGA-354743 Technical (metabolite of CGA 24705, metolachlor)

   Description: light brown solid
   Lot/Batch #: KI-5408/6
   Purity: 98% a.i.
   CAS #: not provided
   Structure: not provided

2. Vehicle and/or positive control

   Distilled water

3. Test animals

   Species: rat
   Strain: HanIbm:WIST
   Age and/or weight at dosing: approximately 8-12 weeks; males: 246-263 g, females: 209-212 g
   Source: BRL Biological Research Laboratories, Wölferstrasse 4, 4414 Füllinsdorf, Switzerland
   Acclimation period: at least 5 days
   Diet: NAFAG No. 890 (NAFAG, Gossau/SG, Switzerland), *ad libitum*
   Water: municipal water, *ad libitum*
   Housing: individually in Macrolon type 3 cages
   Environmental conditions:
   Temperature: 22±3°C
   Humidity: 50±20%
   Air changes: approximately 13-14/hour
   Photoperiod: 12 hour light/dark

B. STUDY DESIGN AND METHODS

1. In life dates

   Start: April 29, 1998; end: May 13, 1998

2. Animal assignment and treatment

   The study was conducted as a limit test using five male and five female rats. The animals were given a single 2000 mg/kg dose of CGA-354743 Technical (5000 mg test material moistened with 5 g of distilled water and at 0.4 g per 100 g body weight) applied to a shaved area (approximately 10% of the body surface) on the back. The

application site was covered with a gauze-lined semiocclusive dressing and fastened with an adhesive elastic bandage. The covering was removed 24 hours later and the site cleaned with lukewarm water. The animals were observed for clinical signs of toxicity daily for 14 days. Mortality was checked twice daily. They were weighed prior to test material application, and on study days 7 and 14. All rats were sacrificed and necropsied.

TABLE 1. Doses, mortality/animals treated

Dose (mg/kg)	Males	Females	Combined
2000	0/5	0/5	0/10

Data taken from p. 13, MRID 44931705.

3. Statistics

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

II. RESULTS AND DISCUSSION

A. MORTALITY

Mortality is give in Table 1. None of the rats died during the study.

The dermal LD_{50} for males, females, and combined was > 2000 mg/kg. This places CGA-354743 Technical (metabolite of CGA 24705, metolachlor) in TOXICITY CATEGORY III.

B. CLINICAL OBSERVATIONS

No remarkable clinical observations or local irritation were noted on any rats.

C. BODY WEIGHT

With the exception of one female that lost weight during the first week, all animals had normal body weight gains.

D. NECROPSY

No observable abnormalities were noted.

E. DEFICIENCIES

None

DATA EVALUATION REPORT

METOLACHLOR ESA
(CGA-354743 TECHNICAL)

STUDY TYPE: PRIMARY EYE IRRITATION - RABBIT [870.2400 (81-4)]
MRID 44931706

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. 00-09C

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Quality Assurance:
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Date: JAN 18 2000

Disclaimer

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Oak Ridge National Laboratory, managed by Lockheed Martin Energy Research Corp. for the U.S. Department of Energy under contract number DE-AC05-96OR22464.

EPA Reviewer: Virginia A. Dobozy, VMD, MPH Virginia A Dobozy, Date 4/17/01
Reregistration Branch I, Health Effects Division (7509C)
EPA Work Assignment Manager: Joycelyn Stewart, PhD Joycelyn Stewart, Date 4/30/01
Toxicology Branch, Health Effects Division (7509C)

DATA EVALUATION RECORD

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

DP BARCODE: D260393 SUBMISSION CODE: S570059
P.C. CODE: 108801 (Metolachlor) TOX. CHEM. NO.: 188DD

TEST MATERIAL (PURITY): CGA-354743 Technical (metabolite of CGA 24705, 98.0% a.i.)

SYNONYMS: not reported

CITATION: Cantoreggi, S. (1998) CGA-354743: Acute eye irritation/corrosion in the rabbit. Novartis Crop Protection AG, Toxicology, 4332 Stein, Switzerland. Laboratory study identification 981041, Novartis No. 646-98, June 19, 1998. MRID 44931706. Unpublished.

SPONSOR: Novartis Crop Protection, Inc., 410 Swing Road, P.O. Box 18300, Greensboro, NC 27419

EXECUTIVE SUMMARY: In a primary eye irritation study (MRID 44931706) 41 mg (0.1 mL weight equivalent) of CGA-354743 Technical (metabolite of CGA 24705, Batch No. KI-5408/6, 98.0% a.i.) was instilled into the conjunctival sac of the left eye of three male and three female rabbits and the eye lids held together for approximately 1 second. The eyes were washed with distilled water for one minute after the 24 hour reading. The contralateral eye of all rabbits served as control. The eyes were scored for ocular irritation according to the OECD/EEC/MAFF scoring system 1, 24, 48, and 72 hours after instillation.

Corneal opacity (grade 1-2) was found in 5/6 rabbits one hour after test material instillation that persisted through 24 hours in 4/6 animals. By 48 hours, 4/6 rabbits had corneal opacity with resolution in 2/6 rabbits by 72 hours, in 1/6 rabbits by day 7 and in 1/6 rabbits by day 10. Iritis (grade 1) was noted on 3/6, 4/6, 2/6, and 2/6 rabbits 1, 24, 48, and 72 hours, respectively, after test material instillation; resolution occurred by 7 days. The test material induced conjunctival redness (grades 1 or 2) in all rabbits within one hour of treatment with resolution in 5/6 rabbits within 7 days and in 1/6 rabbits within 10 days. Conjunctival chemosis (grade 1 or 2) was noted in 6/6 rabbits one hour after treatment which resolved by day 7.

In this study, CGA-354743 Technical was a moderate irritant and is in TOXICITY CATEGORY II for primary eye irritation.

This study is classified as **Acceptable/Guideline** and satisfies the 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: CGA-354743 Technical (metabolite of CGA 24705, metolachlor)

   Description: white solid
   Lot/Batch #: KI-5408/6
   Purity: 98% a.i.
   CAS #: not provided
   Structure: not provided

2. Vehicle

   None

3. Test animals

   Species: rabbit
   Strain: New Zealand White
   Age and weight at dosing: approximately 3-6 months; males: 2260-2490 g; females: 2570-4150 g
   Source: Elevage Scientifique des Dombes, 01400 Chatillon sur Chalaronne, France
   Acclimation period: at least 5 days
   Diet: NAFAG No. 814 (NAFAG, Gossau/SG, Switzerland), *ad libitum*
   Water: municipal water, *ad libitum*
   Housing: individually in cages (Techniplast batteries, Techniplast FRL, Via 1 Maggio 6, 21020 Buguggiate/Varese, Italy)
   Environmental conditions:
   Temperature: 22±3°C
   Humidity: 50±20%
   Air changes: approximately 13-14/hour
   Photoperiod: 12 hour light/dark

B. STUDY DESIGN AND METHODS

1. In life dates

Start: April 28, 1998; end: May 15, 1998

2. Animal assignment and treatment

The test material (0.1 mL weighing 66 mg) was instilled into the conjunctival sac of the left eye of three male and three female rabbits and the eye lids held together for approximately 1 second. The eyes were washed with distilled water for one minute after the 24 hour reading. The contralateral eye of all rabbits served as control. The animals were scored for ocular irritation 1, 24, 48, and 72 hours and 7 and 10 days after instillation according to the OECD/EEC/MAFF scoring system.

II. RESULTS AND DISCUSSION

A. Corneal opacity (grade 1-2)[1] was found in 5/6 rabbits one hour after test material instillation that persisted through 24 hours in 4/6 animals. By 48 hours, 4/6 rabbits had corneal opacity with resolution in 2/6 rabbits by 72 hours, in 1/6 rabbits by day 7 and in 1/6 rabbits by day 10. Iritis (grade 1) was noted on 3/6, 4/6, 2/6, and 2/6 rabbits 1, 24, 48, and 72 hours, respectively, after test material instillation; resolution occurred by 7 days. The test material induced conjunctival redness (grades 1 or 2) in all rabbits within one hour of treatment with resolution in 5/6 rabbits within 7 days and in 1/6 rabbits within 10 days. Conjunctival chemosis (grade 1 or 2) was noted in 6/6 rabbits one hour after treatment which resolved by day 7.

This classifies the test material as a moderate irritant. CGA-354743 Technical (metabolite of CGA 24705, metolachlor) is in TOXICITY CATEGORY II.

B. DEFICIENCIES

None

[1] Using a scale of 0-4 with 0 being no effect and 4 the most severe effect.

DATA EVALUATION REPORT

METOLACHLOR ESA
(CGA-354743 TECHNICAL)

STUDY TYPE: PRIMARY DERMAL IRRITATION - RABBIT [870.2500 (§81-5)]
MRID 44931707

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. 00-09D

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Date: JAN 18 2000

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Signature: H.T Borges
Date: JAN 18 2000

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

Signature: Robert H. Ross
Date: JAN 18 2000

Quality Assurance:
Lee Ann Wilson, M.A.

Signature: L. A. Wilson
Date: JAN 18 2000

Disclaimer

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Oak Ridge National Laboratory, managed by Lockheed Martin Energy Research Corp. for the U.S. Department of Energy under contract number DE-AC05-96OR22464.

EPA Reviewer: Virginia A. Dobozy, VMD, MPH Virginia A Dobozy, Date 4/17/01
Reregistration Branch I, Health Effects Division (7509C)
EPA Work Assignment Manager: Joycelyn Stewart, PhD Joycelyn Stewart, Date 4/25/01
Toxicology Branch, Health Effects Division (7509C)

DATA EVALUATION RECORD

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

DP BARCODE: D260393 SUBMISSION CODE: S570059
P.C. CODE: 108801 (Metolachlor) TOX. CHEM. NO.: 188DD

TEST MATERIAL (PURITY): CGA-354743 Technical (metabolite of CGA 24705, 98.0% a.i.)

SYNONYMS: not reported

CITATION: Cantoreggi, S. (1998) CGA-354743: Acute dermal irritation/corrosion in the rabbit. Novartis Crop Protection AG, Toxicology, 4332 Stein, Switzerland. Laboratory study identification 981040, Novartis No. 644-98, May 1, 1998. MRID 44931707. Unpublished.

SPONSOR: Novartis Crop Protection, Inc., 410 Swing Road, P.O. Box 18300, Greensboro, NC 27419

EXECUTIVE SUMMARY: In a primary dermal irritation study (MRID 44931707) three male and three female adult New Zealand white rabbits were dermally exposed to 0.5 g CGA-354743 Technical (metabolite of CGA 24705, Batch No. KI-5408/6, 98.0% a.i.) on a gauze patch moistened with distilled water for 4 hours on the flank of the animals. The animals were scored 1, 24, 48, and 72 hours after patch removal. Irritation was scored by the method of Draize.

Very slight erythema was noted on 4/6 rabbits (1 male and 3 females) one hour following patch removal with resolution on 2/6 rabbits by 24 hours, on 1/6 rabbits by 48 hours, and on 1/6 rabbits by 72 hours. There was no edema observed. The primary dermal irritation index was 0.29. The female rabbit which had the longest duration of erythema lost weight during the 72-hour period.

In this study, CGA-354743 Technical was essentially nonirritating and is in TOXICITY CATEGORY IV for primary dermal irritation.

This study is classified as **Acceptable/Guideline** and satisfies the 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: CGA-354743 Technical (metabolite of CGA 24705, metolachlor)

   Description: white solid
   Lot/Batch #: KI-5408/6
   Purity: 98% a.i.
   CAS #: not provided
   Structure: not provided

2. Vehicle

   Distilled water

3. Test animals

   Species: rabbit
   Strain: New Zealand White
   Age and weight at dosing: approximately 3-6 months; males: 3130-3460 g; females: 3500-4320 g
   Source: Elevage Scientifique des Dombes, 01400 Chatillon sur Chalaronne, France
   Acclimation period: at least 5 days
   Diet: NAFAG No. 814 (NAFAG, Gossau/SG, Switzerland), *ad libitum*
   Water: municipal water, *ad libitum*
   Housing: individually in cages (Techniplast batteries, Techniplast FRL, Via 1 Maggio 6, 21020 Buguggiate/Varese, Italy)
   Environmental conditions:
   Temperature: 21±2°C
   Humidity: 55±10%
   Air changes: approximately 13-14/hour
   Photoperiod: 12 hour light/dark

B. STUDY DESIGN AND METHODS

1. In life dates

   Start: April 21, 1998; end: April 24, 1998

2. Animal assignment and treatment

Three male and three female animals were given a single 0.5 g dose of CGA-354743 Technical applied to a 2 cm x 3 cm gauze patch (moistened with distilled water) and placed on the clipped site on one flank. A distilled water moistened gauze patch was placed on the other flank as control. The patches were loosely covered with aluminum foil and held in place with adhesive tape. The dressing was removed after four hours and the application site washed with lukewarm water to remove the test material residues. The site was 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 4/6 rabbits (1 male and 3 females) one hour following patch removal with resolution on 2/6 rabbits by 24 hours, on 1/6 rabbits by 48 hours, and on 1/6 rabbits by 72 hours. There was no edema observed. The primary dermal irritation index was 0.29. The female rabbit which had the longest duration of erythema lost weight during the 72-hour period.

CGA-354743 Technical (metabolite of CGA 24705, metolachlor) was essentially nonirritating and is in TOXICITY CATEGORY IV.

B. DEFICIENCIES

None

DATA EVALUATION REPORT

METOLACHLOR ESA
(CGA-354743 TECHNICAL)

STUDY TYPE: DERMAL SENSITIZATION - GUINEA PIG [870.2600 (81-6)]
MRID 44931708

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. 00-09E

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Disclaimer

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

Oak Ridge National Laboratory, managed by Lockheed Martin Energy Research Corp. for the U.S. Department of Energy under contract number DE-AC05-96OR22464.

EPA Reviewer: Virginia A. Dobozy, VMD, MPH Virginia A Dobozy, Date 4/17/01
Reregistration Branch I, Health Effects Division (7509C)
EPA Work Assignment Manager: Joycelyn Stewart, PhD Joycelyn Stewart, Date 4/28/01
Toxicology Branch, Health Effects Division (7509C)

DATA EVALUATION RECORD

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

DP BARCODE: D260393 SUBMISSION CODE: S570059
P.C. CODE: 108801 (Metolachlor) TOX. CHEM. NO.: 188DD

TEST MATERIAL (PURITY): CGA-354743 Technical (metabolite of CGA 24705, 98.0% a.i.)

SYNONYMS: not reported

CITATION: Cantoreggi, S. (1999) CGA-354743: Skin sensitization test in the guinea pig (Buehler test). Novartis Crop Protection AG, Toxicology, 4332 Stein, Switzerland. Laboratory study identification 981042, Novartis No. 1097-99, February 3, 1999. MRID 44931708. Unpublished.

SPONSOR: Novartis Crop Protection, Inc., 410 Swing Road, P.O. Box 18300, Greensboro, NC 27419

EXECUTIVE SUMMARY: In a dermal sensitization study (MRID 44931708) with CGA-354743 Technical (metabolite of CGA 24705, Batch No. KI-5408/6, 98.0% a.i.), 10 young adult male and 10 female guinea pigs were tested using the Buehler Test. An additional five animals/sex served as a vehicle control group and five/sex as a naive control group.

After the first induction, irritation (slight or slight to moderate confluent erythema) at the application site was observed in 13/20 and 18/20 animals at the 24-hour and 48-hour evaluations, respectively. After the second induction, irritation was observed in 17/20 and 18/20 animals at the 24-hour and 48-hour examinations, respectively. After the third induction, all animals had evidence of irritation. The vehicle control animals had no reaction. One female test animal had slight confluent erythema at 48 hours and another female test animal had slight confluent erythema 24 hours after challenge that persisted through 48 hours. No rechallenge was conducted. The flanks of the test animals treated with vehicle had no reaction. The vehicle control and naive control animals had no reaction after challenge. The study report included a positive control study carried out within six months of the current study. The 2-mercaptobenzothiazole positive control and vehicle control animals responded appropriately.

In this study, CGA-354743 Technical was a weak dermal sensitizer.

This study is classified as **Acceptable/Guideline** and satisfies the 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: CGA-354743 Technical (metabolite of CGA 24705, metolachlor)

   Description: white solid
   Lot/Batch #: KI-5408/6
   Purity: 98% a.i.
   CAS #: not provided
   Structure: not provided

2. Vehicle and positive control

   Vehicle: distilled water; positive control: 2-mercaptobenzothiazole

3. Test animals

   Species: guinea pig
   Strain: Himalayan Spotted (GOHI)
   Age and weight at start of treatment: approximately 1-3 months; males: 296-378 g; females: 300-371 g
   Source: BRL Biological Research Laboratories Ltd., 4414 Füllinsdorf, Switzerland
   Acclimation period: 7 days
   Diet: NAFAG No. 845 (NAFAG, Gossau/SG, Switzerland, *ad libitum*
   Water: municipal water, *ad libitum*
   Housing: individually in Macrolon cages type 3 cages
   Environmental conditions:
   Temperature: 22±3°C
   Humidity: 50±20%
   Air changes: approximately 13-14/hour
   Photoperiod: 12 hour light/dark

B. STUDY DESIGN AND METHODS

1. In life dates

   Start: April 29, 1998; end: May 28, 1998

2. Animal assignment and treatment

In a pre-test, one male and one female guinea pig were treated for six hours with four concentrations (10, 30, 50 and 80%) of the test material applied to two sites on each side of the spine. The sites were examined 24 and 48 hours after completion of the application. The 80% concentration produced mild to moderate skin irritation and was selected for induction in the definitive test. The 50% concentration was the highest concentration to produce no irritation and was therefore selected for the challenge application.

In the definitive test, the animals were induced and challenged according to the Buehler Test. The neck-shoulder area on one side of 20 male and 20 female guinea pigs was shaved. For the induction phase, approximately 0.35 mL of 80% test material in distilled water was applied with an occlusive Hilltop Chamber for six hours once each week for three weeks to the clipped side of the test animals (20 animals). For the vehicle control group (10 animals), the chamber contained distilled water. The naive control group animals (10 animals) were not treated. The application site was checked for irritation 24 and 48 hours after each induction treatment. Thirteen days after the third induction, the test animals were challenged with 0.35 mL of 50% test material in distilled water on the clipped flank on the same side as the inductions and with 0.35 mL of distilled water on the clipped flank at the opposite side. The vehicle control and naive control animals were challenged as the test animals. Reactions were scored 24 and 48 hours after inductions and challenge.

II. RESULTS AND DISCUSSION

A. INDUCTION REACTIONS AND DURATION

After the first induction, irritation (slight or slight to moderate confluent erythema) at the application site was observed in 13/20 and 18/20 animals at the 24-hour and 48-hour evaluations, respectively. After the second induction, irritation was observed in 17/20 and 18/20 animals at the 24-hour and 48-hour examinations, respectively. After the third induction, all animals had evidence of irritation. The vehicle control animals had no reaction.

B. CHALLENGE REACTIONS AND DURATION

One female (1/10) test animal had patchy erythema at the test flank 24 hours after challenge that became slight confluent erythema by 48 hours and another female test animal had slight confluent erythema 24 hours after challenge that persisted through 48 hours. The flanks of the test animals treated with vehicle had no reaction. The vehicle control and naive control animals had no reaction after challenge.

CGA-354743 Technical (metabolite of CGA24705, metolachlor) was a weak dermal sensitizer.

C. POSITIVE CONTROL

The study report included a positive control study which was carried out within six months of the current study. The 2-mercaptobenzothiazole positive control and vehicle control animals responded appropriately.

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. However, a rechallenge would firmly determine that the test material was a dermal sensitizer.

E. DEFICIENCIES

A rechallenge will clearly determine if the test material is a dermal sensitizer. It is not clear in the study report whether the inductions and challenge took place at the same site.

DATA EVALUATION REPORT

CGA-354743 (METOLACLOR ESA)
(METABOLITE OF METOLACHLOR)

STUDY TYPE: SUBCHRONIC ORAL TOXICITY FEEDING - DOG
[OPPTS: 870.3150 (§82-1b)]
MRID 44931709

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. 00-09G

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Disclaimer

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

Managed by Lockheed Martin Energy Research, Corp. for the U.S. Department of Energy under Contract No. DE-AC05-96OR22464.

EPA Reviewer: Virginia A. Dobozy, VMD, MPH Virginia A Dobozy, Date 4/17/01
Reregistration Branch I, Health Effects Division (7509C)
EPA Work Assignment Manager: Joycelyn Stewart, PhD Joycelyn Stewart, Date 4/25/01
Toxicology Branch, Health Effects Division (7509C)

DATA EVALUATION RECORD

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

DP BARCODE: D260393
P.C. CODE: 108801 (metolachlor)

SUBMISSION CODE: S570059
TOX. CHEM. NO.: 188DD

TEST MATERIAL (PURITY): CGA-354743 (99% a.i.)

SYNONYMS: none provided

CITATION: Altmann, B. (1999) 3-Month subchronic, comparative oral toxicity study in beagle dogs. Novartis Crop Protection AG, Toxicology, Stein, Switzerland. Laboratory Study Identification 971089, January 25, 1999. MRID 44931709. Unpublished.

SPONSOR: Novartis Crop Protection, Inc., 410 Swing Road, Post Office Box 18300, Greensboro, NC 27419.

EXECUTIVE SUMMARY: In a 90-day subchronic oral toxicity study (MRID 44931709), CGA-354743 technical (Batch Nos. KI-5408/4 and KI-5408/5, 99% a.i.) was administered to 4 purebred beagle dogs/sex/dose by capsule at dose levels of 0, 50, 200, 500, and 1000 mg/kg/day for 13 weeks. An additional group of 4 males and 4 females received parent compound (CGA-77102 technical, Batch No. P.501001, 98.5% a.i.) at 200 mg/kg/day for 13 weeks.

There were no significant treatment related effects on mortality, body weight, food consumption, food conversion ratios, ophthalmological findings, hematology and urinalysis parameters, or gross and histopathological findings. Vomiting did occur at a higher incidence in females treated with 1000 mg/kg/day of CGA-354743. Clinical signs in animals treated with CGA-77102 included vomiting, salivation and hematuria. Mean alkaline phosphatase activity was slightly increased in males receiving 1000 mg/kg/day CGA-354743 at weeks 7 and 13 to levels which were less than double the pretest mean for this group. This finding correlated with slightly increased absolute liver weights, but there were no corresponding histopathological findings, or toxicologically significant increases in other biochemistry parameters. In females, mean ALP activities remained within the reference range for untreated animals and mean GGT activity exceeded the reference range only at week 13 and only for the 500 mg/kg/day CGA-354743 group. Absolute liver weights and liver weights relative to body weights were increased in females receiving 500 and 1000 mg/kg/day. In the absence of corresponding histopathological findings or biologically significant increases in biochemistry parameters consistent with adverse hepatic effects, this finding is not considered toxicologically significant.

Mean ALP and GGT activities were significantly increased in both sexes at weeks 7 and 13 given CGA-77102. In addition, ALT activity of males was increased at weeks 7 and 13. Absolute and relative liver weights were significantly increased in males and females. There were small increases in the incidences and severity of bile duct hyperplasia, perilobular fatty change in the livers of both sexes, and cystic hyperplasia of the gallbladder occurred only in the parent compound group.

The results appear to indicate that CGA-354743 may have effects (vomiting, slight increases in ALT and liver weight) similar to those of its parent compound, CGA-77102; however, at the limit dose, 1000 mg/kg/day, the effects observed were so slight and of questionable toxicological significance in CGA-35743-treated dogs that a definitive comparison of the two compounds cannot be made.

Based on the data presented in this study, the LOAEL was not determined, and the NOAEL was greater than or equal to 1000 mg/kg/day.

This subchronic oral toxicity study in dogs is classified as **Acceptable/Guideline** and satisfies the guideline requirements for a subchronic oral study [OPPTS: 870.3150 (§82-1b)] in dogs since the limit dose was tested.

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

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: CGA-354743 technical

   Description: solid
   Batch Nos.: KI-5408/4 and KI-5408/5
   Purity: 99% a.i. (Both batches)
   Stability of compound: not provided
   CAS #: not provided
   Structure: not provided

2. Parent compound: CGA-77102 technical

   Description: oil
   Batch No.: P.501001
   Purity: 98.5% a.i.
   Stability of compound: not provided
   CAS #: not provided
   Structure: not provided

3. Vehicle: none

4. Test animals

   Species: Dog
   Strain: Purebred beagle
   Age/weight at study initiation: males: 35 to 43 weeks, 11.20-13.70 kg; females: 34 to 49 weeks, 10.40-13.30 kg
   Source: Animal Production, Novartis Pharma AG, 4332 Stein / Switzerland
   Housing: 2/sex/dose in the same kennel. The dogs were chained for feeding.
   Diet: Certified pelleted standard diet (NAFAG 9405 Tox), 350 g/animal daily.
   Water: tap water, *ad libitum*
   Environmental conditions:
   Temperature: minimum room temperature of 15^0 C
   Humidity: not provided
   Air changes: not provided
   Photoperiod: 12 hour light/dark cycle
   Acclimation period: 16 weeks

B. STUDY DESIGN

This study was designed to assess the subchronic oral toxicity potential of CGA-354743 technical when administered by capsule to dogs for 13 weeks and to compare its toxic effects with those of its parent compound, CGA-77102 technical.

1. In life dates - start: September 1, 1997; end: December 4, 1997

2. Animal assignment

Animals were assigned to the test groups in Table 1 by means of a randomized complete block design generated by SAS/STAT procedure PLAN (SAS Institute, Inc.), in order to avoid litter effects and provide homogenous mean body weights among groups.

TABLE 1: Study Design

Test Group	Test Article	Dose Level (mg/kg/day)	Male	Female
Negative Control	None	0	4	4
Low Dose	CGA-354743	50	4	4
Low-Mid Dose	CGA-354743	200	4	4
High-Mid Dose	CGA-354743	500	4	4
High Dose	CGA-354743	1000	4	4
Parent Compound	CGA-77102	200	4	4

Data taken from text table on p. 20, MRID 44931709.

3. Dose selection rationale

Dose selection for the test article and the parent compound was based on the results of a previously conducted rising dose-finding study in dogs (Laboratory Study ID 971088). CGA-354743 technical was administered to 2 male purebred beagle dogs by capsule at dose levels increasing from 350 to 1000 to 2000 mg/kg/day, with the high dose being given for 4 weeks. At 2000 mg/kg/day, the only observed effect was a slight increase in alkaline phosphatase activity.

CGA-77102 technical was administered to 2 male purebred beagle dogs by capsule at dose levels increasing from 50 to 100 to 200 mg/kg/day, with the high dose being given for 4 weeks. Dose levels of 350 or 500 mg CGA-77102 technical/kg/day were administered to either one or two female purebred beagle dogs; the phrasing in the report is unclear. The 350 and 500 mg/kg/day dose levels induced frequent vomiting and were therefore considered too high. The 200 mg/kg/day dose level was well tolerated for four weeks, and a slight increase in alkaline phosphatase activity was observed at this dose in one of the two males.

The doses of CGA-354743 selected for this study were 50 mg/kg/day, which was expected to not induce any effects, 200 mg/kg/day to correspond to the dose selected for the parent compound, 500 mg/kg/day, which was expected not to induce any effects and to be a no-observable-effect level, and 1000 mg/kg/day, which represented a limit dose and was expected to cause minimal effects. The dose of CGA-77102 selected for this study was 200 mg/kg/day, which was expected to cause toxic effects in order to compare the toxicity of the two compounds.

4. Test article preparation and analysis

The test articles were administered in hard-gelatin capsules. Control animals received empty capsules. Capsules were prepared approximately weekly and the dosages were adjusted according to the body weight measurement from the preceding week. No analysis was performed because the test article and parent compound were used as supplied.

5. Statistics

For each time point and parameter a univariate statistical analysis was performed using nonparametric methods to allow for both normal and non-normal data distributions. Groups treated with CGA-354743 technical were compared to the negative control group using Wilcoxon's two-sample test and tested for trends by Jonckheere's test for ordered alternatives. The CGA-77102-treated group and the negative control group were compared using Wilcoxon's two-sample test. Two-sided asymptotic p-values were reported with significance levels of 4% and 1% for Wilcoxon's two-sample test and Jonckheere's test for ordered alternatives, respectively.

C. METHODS

1. Observations

Animals were observed twice daily for mortality, moribundity, and clinical signs.

2. Body weight

Animals were weighed once per week, starting a week before study initiation and throughout the study.

3. Food consumption

Food consumption was measured daily and reported as weekly means. Food consumption ratios (FCR) as "g food/kg body weight/day" were calculated weekly throughout the study.

4. Ophthalmoscopic examination

Ophthalmologic examinations were performed on all animals pre-dosing and towards the end of the treatment period. Mydriaticum™ (Ciba Vision) was used to induce mydriasis, and Novesin™ (0.4%) (Ciba Vision) was used for local anesthesia.

5. Blood was collected from all animals for hematology and clinical analysis pretest and during weeks 7 and 13 using jugular puncture after overnight fasting. The CHECKED (X) parameters were examined.

a. Hematology

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*		
	(Thromboplastin time)		**OTHER**
	(Clotting time)	X	Red cell volume
X	(Prothrombin time)	X	distribution width
			Hemoglobin concentration distribution width

* 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)	X	Triglycerides
	Cholinesterase (ChE)		Serum protein electrophoresis
X	Creatine phosphokinase		
	Lactic acid dehydrogenase (LDH)	X	A/G ratio
X	Serum alanine amino-transferase (also SGPT)*	X	Phospholipids
X	Serum aspartate amino-transferase (also SGOT)*		
X	Gamma glutamyl transferase (GGT)		
	Glutamate dehydrogenase		

* Required for subchronic studies based on Subdivision F Guidelines

6. Urinalysis*

Urine was collected from fasted animals by catheterization pretest and during weeks 7 and 13. The CHECKED (X) parameters were examined.

X		X	
X	Appearance	X	Glucose
	Volume	X	Ketones
X	Specific gravity	X	Bilirubin
X	pH	X	Red blood cells
X	Sediment (microscopic)	X	White blood cells
X	Protein		Nitrate
		X	Urobilinogen

* Not required for subchronic studies.

7. Sacrifice and pathology

Animals were sacrificed at the end of week 13 via injection of T 61 (Hoechst) followed by exsanguination. Detailed necropsies were performed on all animals, and the CHECKED (X) tissues from each animal were preserved in 4% neutral buffered formalin, embedded in paraplast, sectioned, stained with hematoxylin and eosin, and subjected to microscopic examination. The (XX) organs, in addition, were weighed.

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

* Required for subchronic studies based on Subdivision F Guidelines
$^{+}$ Organ weight required in subchronic and chronic studies.
$^{++}$ Organ weight required for non-rodent studies.
T = required only when toxicity or target organ

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II. RESULTS

A. MORTALITY AND CLINICAL SIGNS

No deaths or unscheduled sacrifices occurred during the study. The only treatment related clinical sign observed in animals receiving CGA-354743 technical was an increased number of occurrences of vomiting among females receiving 1000 mg/kg/day (8 occurrences vs. none for controls). These occurred mainly during the first two weeks of the study. Treatment related clinical signs observed from animals receiving CGA-77102 technical included increased numbers of occurrences of vomiting among both males (48 vs. none for controls) and females (111 vs. none for controls). Two females accounted for the majority of the occurrences. Gross hematuria was observed in the kennel of two males several times during weeks 7 and 12.

B. BODY WEIGHT AND WEIGHT GAIN

There were no statistically significant differences in absolute body weights between groups treated with the test material or parent compound and control groups during the study. However, the pretest mean absolute body weight of the male parent compound group was significantly greater than that of controls.

Body weight gain data are given in Table 2. For animals treated with the CGA-354743, there was a lot of variability between mean weight gains throughout the study, but statistical significance was seldom attained. In males receiving the test material, week 2 mean body weight gains were significantly decreased at 50, 200, and 500 mg/kg/day and week 3 mean body weight gains were decreased at 50 mg/kg/day. For females receiving the test material, week 2 mean body weight gains were significantly decreased at all dose levels with a negative trend evident up to the 500 mg/kg/day dose level. No consistent dose-related patterns were observed.

In animals receiving the CGA-77102, there were mean body weight losses in both sexes throughout the study, but statistically significant occurred only during weeks 1-5 and week 8 for males and during weeks 1-3 for females.

C. FOOD CONSUMPTION

1. Food consumption

There were no statistically significant differences in mean food consumption between treated and control groups during the study.

2. Food efficiency

Food efficiency was not determined by the study authors; however, food conversion ratios, which vary inversely as food efficiency, were calculated. There were no statistically significant differences in food conversion ratios between treated and

control groups during the study. Lower mean food consumption ratios were noted during weeks 2-4 for females treated with the parent compound due to the decreased food consumption by one female.

D. OPHTHALMIC EXAMINATION

There were no treatment related effects on ophthalmic examination findings in animals treated with the test material or parent compound.

E. CLINICAL PATHOLOGY

1. Hematology

Hematologic changes among animals treated with the CGA-354743 included increased absolute eosinophil counts among treated males at week 7 (0.158, 0.395, 0.550, 0.448, and 0.550 g/L) and week 13 (0.248, 0.480, 0.663, 0.675, and 0.670 g/L) for controls, 50, 200, 500, and 1000 mg/kg/day groups, respectively. The increase was statistically significant for the 1000 mg/kg/day group. The reference range for this parameter is 0.100-0.550 G/L (Appendix B, page 417).

TABLE 2. Cumulative mean body weight gains (kg) of Beagle dogs administered CGA-354743 technical or CGA-77102 technical by capsule for 13 weeks.

Week	Control	CGA-354743 technical (mg/kg/day)				CGA-77102 tech. (mg/kg/day)
		50	200	500	1000	200
Males						
1	0.125	0.025 (-80)[a]	0.025 (-80)	0.000 (-100)	0.100 (-20)	-0.40*
2	0.175	0.050* (-71)	0.050* (-71)	0.000* (-100)	0.150 (-14)	-0.35*
3	0.200	0.000* (-100)	0.075 (-62)	0.125 (-37)	0.375 (+88)	-0.40*
4	0.250	0.050 (-80)	0.100 (-60)	0.125 (-50)	0.375 (+50)	-0.37*
5	0.250	0.125 (-50)	0.200 (-20)	0.100 (-60)	0.300 (+20)	-0.37*
6	0.175	0.075 (-57)	0.075 (-57)	0.025 (-86)	0.350 (+100)	-0.27
7	0.175	0.000 (-100)	-0.05 (-129)	0.000 (-100)	0.250 (+43)	-0.35
8	0.175	0.150 (-14)	0.125 (-29)	0.050 (-71)	0.250 (+43)	-0.35*
9	0.100	0.025 (-75)	-0.02 (-120)	0.000 (-100)	0.225 (+125)	-0.27
10	0.100	0.075 (-25)	0.050 (-50)	0.025 (-75)	0.250 (+150)	-0.35
11	0.200	0.125 (-38)	0.200	0.075 (+63)	0.500 (+150)	-0.27
12	0.125	0.100 (-20)	0.075 (-40)	0.025 (-80)	0.425 (+240)	-0.30
13	0.000	-0.02	-0.07	-0.02	0.300	-0.42
Females						
1	0.075	0.025 (-67)	0.050 (-33)	0.000 (-100)	0.050 (-33)	-0.30*
2	0.250	0.025* (-90)	0.000* (-100)	-0.05*# (-120)	0.100* (-60)	-0.32*
3	0.150	0.025 (-83)	0.100 (-33)	0.000 (-100)	0.075 (-50)	-0.37*
4	0.225	0.050 (-77)	0.125 (-44)	0.075 (-67)	0.175 (-22)	-0.45
5	0.300	0.050 (-83)	0.275 (-8)	0.000 (-100)	0.125 (-58)	-0.62
6	0.150	-0.02 (-113)	0.100 (-33)	0.100 (-33)	0.200 (+33)	-0.50
7	0.150	-0.02 (-113)	0.050 (-67)	0.075 (-50)	0.125 (-17)	-0.52
8	0.225	0.025 (-89)	0.075 (-67)	0.125 (-44)	0.150 (-33)	-0.37
9	0.100	0.000 (-100)	0.025 (-75)	0.025 (-75)	0.100	-0.42
10	0.100	0.000 (-100)	0.075 (-25)	-0.00 (-100)	0.150 (+50)	-0.32
11	0.200	0.100 (-50)	0.125 (-38)	0.000 (-100)	0.125 (-38)	-0.12
12	0.200	0.050 (-75)	0.075 (-63)	0.050 (-75)	0.225 (+13)	-0.20
13	0.100	-0.05 (-150)	0.000 (-100)	-0.02 (-120)	0.200 (+100)	-0.25

Data taken from Table 9.3, pp. 56-58, MRID 44931709.

* Significantly different than controls; $p<0.04$.

Significant negative trend from control up to the flagged dose level; $p<0.01$.

[a] Number in parenthesis equals percent greater than or less than control, calculated by reviewer.

Other statistically significant inter-group differences were observed but were not considered to be treatment related because there was no dose-response pattern, the magnitudes of the changes were too small to be toxicologically significant, and/or the values were not appreciably different from pretest values.

2. Clinical chemistry

Selected clinical chemistry parameters are summarized in Tables 3 and 4. Alkaline phosphatase (ALP) activities were increased at week 7 in males receiving 1000 mg/kg/day and at week 13 in males receiving 500 and 1000 mg/kg/day CGA-354743. ALP activities of treated females remained within the reference range, although at 500 and 1000 mg/kg/day, ALP activities were slightly higher than controls. Gamma-glutamyl transpeptidase (GGT) activities were slightly increased in males at 1000 mg/kg/day CGA-354743 for week 7 and week 14, however, these values were within the reference range for untreated animals. In females, GGT activity was only increased above the reference range for the 500 mg/kg/day group and only at week 14.

For animals treated with CGA-77102, ALP and GGT activities were significantly increased in both sexes at week 7 and week 14. Albumin levels were decreased below the reference range in males at week 7 and week 13 while the mean globulin concentration was increased above the reference range for males at both time intervals. For males, mean ALT activity was increased at weeks 7 and 13 as compared with controls and referenced range values.

Other statistically significant inter-group differences were observed but were not considered to be treatment related and/or biologically significant because there was no dose-response pattern, the magnitudes of the changes were too small to be toxicologically significant, the values were not appreciably different from pretest values, or the values fell within the provided reference range for untreated animals.

TABLE 3. Selected mean clinical chemistry parameters in male Beagle dogs administered CGA-354743 technical or CGA-77102 technical by capsule for 13 weeks.

Clinical Chemistry Parameter	Week	Treatment group						Reference Range
		Control	CGA-354743 tech. (mg/kg/day)				CGA-77102 tech. 200 mg/kg/day	
			50	200	500	1000		
ALP (U/L)	-1	85.95	91.30	91.60	77.95	82.75	82.33	56.60-137.7
	7	74.80	77.10	88.65	100.6	159.4#	265.4*	
	13	68.35	83.00	97.23	110.2*	141.4*#	308.7*	
GGT (U/L)	-1	2.800	3.100	2.950	3.025	3.275	2.225	0-5.4
	7	2.750	2.825	3.125	2.900	4.150*	6.350*	
	13	3.575	4.175	4.350	4.350	5.275*#	13.01*	
Albumin (g/L)	-1	31.31	32.34	32.28	32.13	32.41	32.95	30.53-36.14
	7	32.62	33.54	33.93	31.92	31.79	30.90	
	13	32.28	33.66	33.43	32.31	31.71	29.95*	
Globulin (g/L)	-1	24.20	24.68	24.53	25.47	25.78	24.26	21.45-28.22
	7	25.01	25.35	24.73	26.83	26.20	29.40*	
	13	26.73	27.48	26.17	28.39	27.41	29.52	
A/G ratio	-1	1.310	1.318	1.318	1.268	1.260	1.360	1.160-1.560
	7	1.303	1.335	1.375	1.195	1.223	1.058*	
	13	1.223	1.230	1.283	1.153	1.163	1.015	
ALT (U/L)	-1	56.65	67.83	56.30	55.48	46.20	51.78	36.30-61.60
	7	45.45	56.25	48.50	41.23	59.14	71.58	
	13	57.25	55.33	74.76	47.13	51.93	127.1	
Potassium (mmol/L)	-1	4.420	4.495	4.403	4.273	4.463	4.358	4.010-4.830
	7	4.205	4.400	4.385	4.345	4.383	3.895	
	13	4.281	4.245	4.149	4.075	4.173	3.858	

Data taken from Tables 9.10, 9.11, and 11.4 , pp. 132-139, 140-171, and 418-419, respectively, MRID 44931709.
* Significantly different than controls; $p<0.04$.
# Significant positive or negative trend from control up to the flagged dose level; $p<0.01$.

TABLE 4. Selected mean clinical chemistry parameters in female Beagle dogs administered CGA-354743 technical or CGA-77102 technical by capsule for 13 weeks.

Clinical Chemistry Parameter	Week	Treatment Group						Reference Range
		Control	CGA-354743 tech. (mg/kg/day)				CGA-77102 tech. 200 mg/kg/day	
			50	200	500	1000		
ALP (U/L)	-1	78.88	91.38	73.98	82.23	66.38	72.70	48.7-123.6
	7	63.75	77.33	82.63	107.6*	116.0	211.9*	
	13	62.08	79.53	79.15	112.7*#	120.0	256.1*	
GGT (U/L)	-1	2.825	3.025	2.975	3.025	2.050	2.725	0- 5.1
	7	2.825	2.900	2.975	3.525	2.050	5.250*	
	13	1.800	3.950	3.750	5.450*#	4.650	8.525*	
Albumin (g/L)	-1	34.82	34.30	35.13	33.30	34.45	33.27	31.71-36.89
	7	35.45	35.14	35.09	32.92*	31.80*#	31.80*	
	13	36.10	35.48	35.93	33.20*	33.07*#	32.07*	
Globulin (g/L)	-1	25.00	23.22	24.60	25.41	23.88	24.44	20.30-26.95
	7	24.23	21.09	22.90	25.63	24.77	25.53	
	13	26.76	22.62*	24.59	28.38	26.20	29.10	
A/G ratio	-1	1.418	1.488	1.435	1.315	1.453	1.360	1.270-1.720
	7	1.478	1.678	1.538	1.288	1.295	1.253	
	13	1.363	1.573	1.468	1.168	1.273	1.118	

Data taken from Tables 9.10, 9.11, and 11.4 , pp. 132-139, 140-171, and 418-419, respectively, MRID 44931709.

* Significantly different than controls; $p<0.04$.
# Significant positive or negative trend from control up to the flagged dose level; $p<0.01$.

F. URINALYSIS

There were no treatment related effects on urinalysis parameters in animals treated with the test material or parent compound.

G. SACRIFICE AND PATHOLOGY

1. Organ weight

Selected organ weight data are given in Table 5. In animals treated with CGA-354743, absolute liver weights were increased in males at 1000 and females at 500 and 1000 mg/kg/day with a positive trend ($p<0.01$) evident in males at 1000 mg/kg/day. Relative liver weights were increased in females at 500 and 1000 mg/kg/day with a positive trend ($p<0.01$) evident at 1000 mg/kg/day. In animals treated with CGA-77102, absolute and relative liver weights were significantly increased in males and females. There were no other treatment related effects on organ weights.

TABLE 5. Selected mean organ weight data of Beagle dogs administered CGA-354743 technical or CGA-77102 technical by capsule for 13 weeks.						
Parameter	**Control**	**CGA-354743 technical (mg/kg/day)**				**CGA-77102 tech. (mg/kg/day)**
		50	**200**	**500**	**1000**	**200**
Males						
Liver weight (g)	344.2	314.8	344.8	373.4	375.1# (109)	470.2* (137)
Relative liver weight (%)	30.81	26.42	29.13	31.82	31.78	40.10 (130)
Females						
Liver weight (g)	288.1	310.0	302.8	338.0 (117)	330.9 (115)	429.5* (149)
Relative liver weight (%)	26.03	27.44	27.95	29.55 (114)	30.12# (116)	40.02* (154)

Data taken from Tables 9.14-9.15, pp. 194-197, respectively, MRID 44931709.
* Significantly different than controls; $p<0.05$.
# Significant positive or negative trend from control up to the flagged dose level; $p<0.01$.
Number in parenthesis equals percent of control calculated by reviewer.

2. Gross pathology

There were no treatment related gross necropsy findings in the animals treated with the test material or the parent compound. Mottled lungs were observed in 7/24 males and 3/24 females with a random distribution among groups.

3. Microscopic pathology

Selected histopathology data are given in Table 6. There were no histopathology findings in the animals treated with CGA-354743. In the CGA-77102 group, there were small increases in the incidences and severity of bile duct hyperplasia and perilobular fatty change in the livers of both sexes as compared to controls and animals treated with the CGA-354743. Cystic hyperplasia of the gallbladder occurred only in the CGA-77102 group. Although, the incidence and severity of these findings were considered to be within the normal ranges for dogs of this age group, these findings were considered to be treatment related because they correlated with increased liver weights and changes in the biochemical profile. An unusual pattern of multifocal spermatic granulomata was observed in the testes of one male dog treated with the CGA-77102; it could not be determined whether this finding was treatment related. Acute bronchopneumonia (Grades 1-3) or chronic bronchopneumonia (Grades 1-2) were observed in male and females dogs representing all groups except the parent compound group. This finding was clearly not treatment related.

TABLE 6. Incidences of selected histopathology findings in Beagle dogs administered CGA-354743 technical or CGA-77102 technical by capsule for 13 weeks.

Histopathology finding	Control	CGA-354743 technical (mg/kg/day)				CGA-77102 tech. (mg/kg/day)
		50	200	500	1000	200
Males						
Liver-						
Perilobular fatty change	0/4	0/4	0/4	0/4	0/4	4/4
Bile duct hyperplasia	1/4	0/4	0/4	1/4	1/4	4/4
Gallbladder-						
Cystic hyperplasia	0/4	0/4	0/4	0/4	0/4	2/4
Lungs-bronchopneumonia						
Acute	0/4	1/4	0/4	2/4	1/4	0/4
Chronic	0/4	1/4	1/4	0/4	0/4	0/4
Females						
Liver-						
Perilobular fatty change	0/4	1/4	0/4	0/4	0/4	2/4
Bile duct hyperplasia	0/4	1/4	1/4	2/4	0/4	4/4
Gallbladder-						
Cystic hyperplasia	0/4	0/4	0/4	0/4	0/4	3/4
Lungs-bronchopneumonia						
Acute	1/4	0/4	0/4	0/4	0/4	0/4
Chronic	0/4	1/4	0/4	0/4	2/4	0/4

Data taken from Pathology Report Summary Tables, pp. 467-469, MRID 44931709.

III. DISCUSSION

A. DISCUSSION

CGA-354743 technical: There were no significant treatment related effects on mortality, body weight, food consumption, food conversion ratios, ophthalmological findings, urinalysis parameters, or gross and histopathological findings. Eight occurrences of vomiting were observed among females at 1000 mg/kg/day. Vomiting is common among research dogs; however, because it occurred in the high dose animals, a treatment-related effect cannot be ruled out. Slight eosinophilia was observed in males at week 13 at 200, 500, and 1000 mg/kg/day with statistical significance being achieved for the 1000 mg/kg/day group. This finding was probably treatment related, as no findings consistent with other causes of eosinophilia (such as ecto- or endoparasites, food allergies, or allergic dermatitis) were identified among clinical signs, or gross and microscopic pathology findings. However, this finding is of questionable toxicological

significant. Males at 1000 mg/kg/day had increased mean ALP activities at weeks 7 and 13 which were both less than double the pretest mean for this group. This finding correlated with slightly increased absolute liver weights (9% greater than controls), but there were no corresponding histopathological findings, or increases in other biochemistry parameters (ALT, AST, GGT, bilirubin) which might indicate a significant adverse hepatic effect. Although GGT activity was significantly increased in males at 1000 mg/kg/day at week 7 and week 13, these values were within the reference range for untreated animals, and this finding is therefore not considered to be biologically significant. In females, mean ALP activities remained within the reference range for untreated animals, and mean GGT activity exceeded the reference range only at week 13 and only for the 500 mg/kg/day group. The study author also mentions decreased mean albumin concentrations and increased mean globulin concentrations in females at 500 and 1000 mg/kg/day. The only values outside the reference ranges were week 13 mean globulin concentration and A/G ratio for the 500 mg/kg/day group, not the high dose group. Absolute liver weights were increased in females at 500 and 1000 mg/kg/day and relative liver weights were increased in females at 500 and 1000 mg/kg/day with a positive trend evident at 1000 mg/kg/day. In the absence of corresponding histopathological findings or biologically significant increases in biochemistry parameters consistent with adverse hepatic effects, this finding is of questionable toxicological significance.

CGA-77102 technical (200 mg/kg/day): There were no significant treatment related effects on mortality, ophthalmological findings, urinalysis parameters, or gross necropsy findings. Clinical signs included vomiting, salivation, and hematuria. Food consumption was transiently decreased in one female. Both groups exhibited mean weight loss throughout the study, although there was no effect on mean absolute body weights. Mean ALP and GGT activities were significantly increased in both sexes at weeks 7 and 13 . Albumin levels were decreased below the reference range while globulin concentrations were increased in males at weeks 7 and 13. Globulin concentrations were increased above the reference range for females at week 13 only. For males, mean ALT activity was increased at weeks 7 and 13 as compared with controls and the reference range for untreated animals. Absolute and relative liver weights were significantly increased in males and females. There were small increases in the incidences of bile duct hyperplasia and perilobular fatty change in the livers of both sexes as compared to controls and animals treated with CGA-354743, and cystic hyperplasia of the gallbladder occurred only in the parent compound group. These findings were considered treatment related because they correlate with increased liver weights and changes in the biochemical profile.

A comparison of the effects of the two compounds indicates that CGA-354743 may have effects similar to those of its parent compound, CGA-77102; however, the data indicate that CGA-354743 needs substantially higher dose levels than that of the parent compound to obtain similar adverse effects. A definitive comparison of the two compounds cannot be made based on the results of this study.

Based on the data presented in this study, the LOAEL for CGA-354743 technical was not determined and the NOAEL was greater than or equal to the limit dose of 1000 mg/kg/day.

This subchronic oral toxicity study in dogs is classified as **Acceptable/Guideline** and satisfies the guideline requirements for a subchronic oral study since the limit dose was tested [OPPTS: 870.3150 (§82-1b)] in dogs.

B. STUDY DEFICIENCIES

There were no major deficiencies in the conduct of this study; however, the following minor deficiencies were noted. Animals were assigned to test groups by means of a randomized complete block design intended to avoid litter effects and provide homogenous mean body weight among groups. This was done immediately after the animals arrived at the lab, and when the study began 16 weeks later, the mean body weight of the male CGA-77102 group was significantly greater than that of controls. At initiation of dosing, males were up to 43 weeks old and females were up to 49 weeks old; however, the guideline specifies that dosing should commence "not later than 9 months of age." Housing the animals in groups of two made it impossible to accurately determine which and how many animals were exhibiting the clinical signs of vomiting, diarrhea, and hematuria. A brief description of the histopathology grading criteria should have been included for findings which were assigned a grade. Also, although the high incidence bronchopneumonia was not treatment related, it should have been mentioned and addressed by the study author.

DATA EVALUATION REPORT

CGA-354743

STUDY TYPE: SUBCHRONIC ORAL TOXICITY FEEDING - RAT
[OPPTS 870.3100 (§82-1a)]
MRID 44931710

Prepared for

Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
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Prepared by

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Disclaimer

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

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Reregistration Branch I, Health Effects Division (7509C)
EPA Work Assignment Manager: Joycelyn Stewart, PhD Joycelyn Stewart, Date 6/8/2001
Toxicology Branch, Health Effects Division (7509C)

DATA EVALUATION RECORD

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

DP BARCODE: D260393 SUBMISSION CODE: S570059
P.C. CODE: 108801 TOX. CHEM. NO.: 188DD

TEST MATERIAL (PURITY): CGA-354743 (a.i. 98%)

SYNONYMS: Metolachlor ESA (degredate of metolachlor)

CITATION: Bachmann, M. (1999) CGA-354743: Final report. 3-month oral toxicity study in rats (Administration in food). Novartis Crop Protection AG, Toxicology, 4332 Stein, Switzerland. Study # 971142, Novartis # 1187-98. January 26, 1999. MRID 44931710. Unpublished.

SPONSOR: Novartis Crop Protection, Inc., 410 Swing Road, P.O. Box 18300, Greensboro, NC 27419.

EXECUTIVE SUMMARY: In a 90-day subchronic oral toxicity limit study (MRID 44931710), groups of 10 male and 10 female Crl: CD BR rats were given CGA-354743 (Lot/Batch # KI-5408/6, 98% a.i.) administered in the diet at concentrations of 0, 360, 1200, 6000, or 20,000 ppm. These concentrations were equivalent to 0, 25.1, 86.2, 427.0 or 1545.0 mg/kg/day for males and 0, 28.4, 98.3, 519.0 and 1685.0 mg/kg/day for females. An additional 10 male and 10 female rats were given CGA-77102 (s-Metolachlor)(Lot/Batch # P.501001, 98.5% a.i.) administered in the diet at 5000 ppm (equivalent to 429 mg/kg/day for males and 563 mg/kg/day for females). The study was designed to assess the subchronic oral toxicity of CGA-354743 technical and to compare its toxic effects with those of its parent compound, CGA-77102 technical.

No deaths or clinical signs of toxicity occurred during this study. In addition, no statistically significant changes in body weight, body weight gain, food consumption, food efficiency, ophthalmologic examination, urinalysis, or histopathology was reported for animals fed CGA-354743. Limited and sporadic statistically significant changes in hematology, clinical chemistry, water intake and organ weight data were not dose-dependent, and were of questionable toxicological and biological importance.

Dietary exposure to CGA-77102 produced a statistically significant decreased body weight gain (-20%, $p \leq 0.01$) in males during week 1 only. Females exposed to CGA-77102 showed decreased body weight gain (-19%) by week 13, but these changes were not statistically significant. The food efficiency of rats fed CGA-77102 was decreased relative to their respective

control animals. Male and female rats had increased absolute and relative liver weights. These results are consistent with a mild liver hypertrophy in females.

Based on the data presented in this study, the NOAEL is ≥20,000 ppm (1543 mg/kg/day and 1685 mg/kg/day for females) for CGA-354743. A LOAEL could not be established. At 5000 ppm (429 mg/kg/day in males and 563 mg/kg/day in females) CGA-77102 , there evidence of decreased body weight gain and food efficiency, increased absolute and relative liver weights and an increased incidence of hepatic centrilobular hypertrophy, although the effects were mild.

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

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

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: CGA-354743

Description: Solid
Lot/Batch #: KI-5408/6
Purity: 98% a.i.
Stability of compound: 5 weeks at room temperature
CAS #: not reported
Structure: not available

2. Vehicle and/or positive control

none

3. Test animals

Species: Rat
Strain: Crl: CD BR
Age/weight at study initiation: males: 4 weeks, 127 -174 g; females: 4 weeks, 104 - 149 g
Source: Charles River Deutschland GmbH, Sulzfeld, Germany
Housing: Individually, Macrolon type 3 cages
Diet: Certified standard diet (NAFAG # 8900), *ad libitum*
Water: tap water, *ad libitum*

4. Environmental conditions

Temperature: 20-24°C
Humidity: 45-65%
Air changes: 16-20/hour
Photoperiod: 12 hour light/dark cycle
Acclimation period: 11 days

B. STUDY DESIGN

1. In life dates - start: 02/23/98 end: 05/29/98

2. Animal assignment

Animals were assigned to one of 6 groups based on body weights using a computer randomization program (Table 1). Ten rats/sex/dose were used except for the control groups where 20/sex were used. Of the treated rats, four groups were given varying

concentrations of CGA-354743, and one group was fed CGA-77102 tech. (S-Metolachlor, batch # P.501001, a.i.% 98.5%). CGA-77102 was used to allow direct comparison of the toxicity to CGA-354743 (a major soil metabolite of CGA-77102).

TABLE 1. Study Design

Group	Group number	Number of animals	Dose (mg/kg/day)[a]	
			Males	Females
CGA-354743				
control	1	20 ♂/20♀	0.0	0.0
360 ppm	2	10 ♂/10♀	25.1	28.4
1200 ppm	3	10 ♂/10♀	86.2	98.3
6000 ppm	4	10 ♂/10♀	427.0	519.0
20000 ppm	5	10 ♂/10♀	1545.0	1685.0
CGA-77102				
5000 ppm	6	10 ♂/10♀	429.0	563.0

[a] Dose level (mg/kg/day) was taken from p. 43; MRID 44931710.

3. Dose selection rationale

Doses were selected by the sponsor based on previous subchronic toxicity studies with CGA-77102. The lowest dose, 360 ppm, was intended as the NOAEL, 1200 ppm to cause no or minimal adverse effects, 6000 ppm to cause minimal adverse effects, and 20,000 ppm to cause observable adverse effects with no or few fatalities. CGA-77102 was tested on an equimolar basis, comparing 5000 ppm CGA-77102 with 6000 ppm CGA-354743.

4. Test diet preparation and analysis

The appropriate amount of CGA-354743 was weighed (without adjustment for purity) and mixed with pulverized diet containing approximately 25% water. After mixing, pellets were formed and air dried. CGA-77102 (an oily liquid) was weighed and 130 g dissolved in 500 mL acetone. A premix diet was made using an aliquot of this solution added to a fixed amount of diet. The acetone was removed under vacuum at 22°C and the premix mixed with a fixed diet quantity to yield appropriate treatment concentrations. Fresh diets were prepared monthly and stored at room temperature.

Analyses were performed on all test diets used for treatment weeks 1-5 and 10 to the end of the study. Batches used for weeks 1 - 5 were analyzed for homogeneity using samples taken at the beginning, middle and end of the pelleting process. Stability analyses were performed after 5 weeks storage at room temperature from pretest preparations at 100, 1000, 10,000 and 20,000 ppm.

Results –
Homogeneity: CGA-354743 concentrations ranged from 96.4 - 102.8% of nominal.

Stability: CGA-354743 was stable for 5 weeks at room temperature. Concentrations varied between -10.4% and +1.4% of the mean values calculated from the homogeneity determinations.

Concentration: Mean concentrations of 106%, 105%, 108%, 106% and 106% of nominal for groups 2-6, respectively, were calculated.

The analytical data was sufficient to establish that the mixing procedure was adequate and the variance between nominal and actual dosage to the animals was acceptable.

5. Statistics

Body weight, food consumption, laboratory data and organ weight data were analyzed using univariate analyses at each time point. Each treatment group was compared to the control group either by Lepage's or by Wilcoxon's two-sample test and tested for increasing or decreasing trends from control up to the respective dose group by Jonckeere's test for ordered alternatives.

C. METHODS

1. Observations

Animals were observed twice daily for mortality and moribundity.

2. Body weight

Animals were weighed at study initiation and once per week throughout the study.

3. Food consumption, compound intake and water intake

Food consumption, compound consumption and water intake were calculated weekly.

4. Blood was collected from all animals at the end of week 13 via orbital sinus puncture after overnight fasting for hematology and clinical biochemical analysis. The CHECKED (X) parameters were examined.

a. Hematology

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*		Reticulocyte count
	Blood clotting measurements*	x	Red cell volume distribution width (RDW)
x	(Thromboplastin time)	x	Hemoglobin concentration distribution width (HDW)
	(Clotting time)		Methemoglobin (metHb)
x	(Prothrombin time)		
x	(Fibrinogen)		

* 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)	x	Triglycerides
	Cholinesterase (ChE)		Serum protein electrophores
	Creatine phosphokinase	x	A/G ratio
	Lactic acid dehydrogenase (LDH)		
x	Serum alanine amino-transferase (also SGPT)*		
x	Serum aspartate amino-transferase (also SGOT)*		
x	Gamma glutamyl transpeptidase		
	Glutamate dehydrogenase		

* Required for subchronic studies based on Subdivision F Guidelines

5. Urinalysis

Urine was collected overnight from individual rats housed in metabolism cages. The CHECKED (X) parameters were examined.

Physical/chemical examinations			
x	Volume	x	Ketones
x	Relative Density	x	Urobilinogen
x	Color	x	Bilirubin
x	pH	x	Erythrocytes
x	Protein	x	Leukocytes
x	Glucose		

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6. Ophthalmologic examination

Control and highest dose animals were examined ophthalmologically prior to dosing and during week 13. Examinations included ophthalmoscopic inspection and induction of mydriasis with Mydriaticum™.

7. Neurotoxicity screening

Neurotoxicity screening was not performed.

8. Sacrifice and pathology

Animals were sacrificed at the end of week 13 via carbon dioxide anesthesia and exsanguination following overnight fast. Necropsies were done on all animals and tissues from each animal were preserved in neutral buffered 4% formalin. After formalin fixation, tissues were embedded in paraffin, sectioned at 3-5 microns, stained with hematoxylin and eosin, and subjected to microscopic analyses. The CHECKED (X) tissues were collected and examined histologically. The (XX) organs, in addition, were weighed.

	DIGESTIVE SYSTEM		CARDIOVASC./HEMAT.		NEUROLOGIC
x	Tongue	x	Aorta*	xx	Brain*
x	Salivary glands*	xx	Heart*	x	Periph. nerve*
x	Esophagus*	x	Bone marrow*	x	Spinal cord (3 levels)[T]
x	Stomach*	x	Lymph nodes*	x	Pituitary*
x	Duodenum*	xx	Spleen*	x	Eyes (optic n.)[T]
x	Jejunum*	xx	Thymus*		
x	Ileum*				GLANDULAR
x	Cecum*		UROGENITAL	xx	Adrenal gland*
x	Colon*	xx	Kidneys*+	x	Lacrimal gland[T]
x	Rectum*	x	Urinary bladder*	x	Mammary gland[T]
xx	Liver*+	xx	Testes*+	x	Parathyroids*
x	Pancreas*	xx	Epididymides	xx	Thyroids*
		x	Prostate		
	RESPIRATORY	x	Seminal vesicle		OTHER
x	Trachea*	xx	Ovaries	x	Bone
x	Lung*	x	Uterus*	x	Skeletal muscle
x	Nose	x	Vagina	x	Skin
	Pharynx			x	All gross lesions and masses*
	Larynx			x	Harderian glands
				x	Zymbal's glands

* Required for subchronic studies based on Subdivision F Guidelines
+ Organ weight required in subchronic and chronic studies.
T = required only when toxicity or target organ

II. RESULTS

A. OBSERVATIONS

No deaths or clinical signs of toxicity were reported during the study.

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B. BODY WEIGHT AND WEIGHT GAIN

Body weight and body weight gain were not affected by CGA-354743 treatment. Dietary exposure to CGA-77102 produced a statistically significant decreased body weight gain (-20%, $p \leq 0.01$) in males during week 1 only. Females exposed to CGA-77102 showed decreased body weight gain (-19%) by week 13, but these changes were not statistically significant. Data are presented in Tables 2a and 2b.

Table 2a: Mean body weight (g) and mean body weight gain in males treated with CGA-34743 or CGA-77102

	Dose Levels (ppm)					
	CGA-354743					CGA-77102
	0	360	1200	6000	20000	5000 ppm
Mean Body Weight (g)						
Week -1	148.8 ± 10.26	150.00 ± 11.11	150.7 ± 9.47	151.6 ± 13.20	153.0 ± 12.43	149.6 ± 12.26
Week 1	208.0 ± 10.03	211.8 ± 8.61	212.7 ± 10.70	209.6 ± 15.89	210.2 ± 13.34	196.9 ± 15.49
Week 13	491.1 ± 47.69	509.4 ± 25.81	515.1 ± 38.08	498.0 ± 51.72	506.5 ± 48.80	476.0 ± 53.79
Cumulative Mean Body Weight Gain (g)						
Week 1	59.21 ± 6.68	61.81 ± 5.61	61.99 ± 5.08	58.05 ± 3.71	57.18 ± 4.64	47.30 ± 8.15*
Week 13	342.3 ± 45.89	359.4 ± 27.88	364.4 ± 40.02	346.4 ± 46.16	353.5 ± 48.63	326.5 ± 55.32

Extracted from Tables 8.7 (pages 72-75) and 8.9 (pages 82-85) of MRID 44931710

Table 2b: Mean body weight (g) and mean body weight gain in females treated with CGA-34743 or CGA-77102

	Dose Levels (ppm)					
	CGA-354743					CGA-77102
	0	360	1200	6000	20000	5000 ppm
Mean Body Weight (g)						
Week -1	124.6 ± 9.00	125.8 ± 6.02	128.0 ± 10.73	124.5 ± 10.58	124.3 ± 9.11	125.6 ± 12.19
Week 1	154.0 ± 13.48	156.5 ± 6.60	158.1 ± 13.80	156.2 ± 14.02	151.5 ± 11.19	147.4 ± 17.08
Week 13	273.0 ± 26.98	284.4 ± 25.39	269.1 ± 30.64	272.2 ± 27.18	264.6 ± 28.20	247.3 ± 34.69
Cumulative Mean Body Weight Gain (g)						
Week 1	29.34 ± 5.74	30.71 ± 4.30	30.12 ± 4.91	31.66 ± 4.36	27.21 ± 4.76	21.80 ± 6.65
Week 13	148.4 ± 21.76	158.6 ± 25.19	141.1 ± 21.06	147.6 ± 20.03	140.3 ± 23.29	121.7 ± 25.84

Extracted from Tables 8.7 (pages 76-79) and 8.9 (pages 86-89) of MRID 44931710

C. FOOD CONSUMPTION, COMPOUND INTAKE AND WATER INTAKE

1. Food consumption

Mean food consumption (g/animal/week) and food consumption ratios (g food/kg body weight/day) in animals fed CGA-354743 were not significantly changed throughout the study.

2. Compound consumption

Achieved doses were generally close to nominal in all cases (Table 3).

TABLE 3. Mean compound consumption of CGA-354743 and CGA-77102

Group Number	Males		Females	
	Target Dietary Level mg/kg/day	Achieved Dietary Level mg/kg/day (% nominal)	Target Dietary Level mg/kg/day	Achieved Dietary Level mg/kg/day (% nominal)
Group 1	0	0	0	0
Group 2	25.1	26.6 (106%)	28.4	30.1 (106%)
Group 3	86.2	90.6 (105%)	98.3	103.0 (105%)
Group 4	427.0	461.0 (108%)	519.0	560.0 (108%)
Group 5	1545.0	1638.0 (106%)	1685.0	1786.0 (106%)
Group 6	429.0	454.0 (106%)	563.0	597.0 (106%)

Data taken from p. 43; MRID 44931710.

3. Food efficiency

Food efficiency of rats fed CGA-354743 was similar to that of control rats. The food efficiency of rats fed CGA-77102 was decreased relative to their respective control animals (Table 4).

TABLE 4. Mean overall group food efficiency (weeks 1-13)[a]		
Treatment group	**Males**	**Females**
Group 1 - Control	13.7	8.5
Group 2 - CGA-354743	14.9	9.5
Group 3 - CGA-354743	14.7	8.5
Group 4 - CGA-354743	14.4	8.2
Group 5 - CGA-354743	13.2	8.2
Group 6 - CGA-77102	11.8	6.1

[a]Data calculated by the reviewer: $\frac{\text{Overall body weight gain (g)}}{\text{Total food consumption} \times 100}$

4. Water Intake

The water consumption of male and female rats fed 20,000 ppm CGA-354743 was statistically increased approximately 25% relative to their respective control rats throughout the study. No other significant differences in water consumption for the remaining groups was found.

D. CLINICAL PATHOLOGY

1. Hematology

Although sporadic statistically significant changes were found in certain hematological parameters, they were of little toxicological or biological relevance and were within the reference values for the laboratory. These included marginally increased counts for white blood cells, eosinophils, and lymphocytes for females receiving 6000 ppm (519 mg/kg/day) CGA-354743 and the MCV of male rats receiving 6000 ppm (427.0 mg/kg/day).

2. Clinical chemistry

Sporadic statistically significant changes for a limited number of clinical chemistry parameters were reported. These included phosphorous for males receiving 20,000 ppm (1545 mg/kg/day) and females receiving 6000 ppm (519 mg/kg/day), and urea for males receiving 20,000 ppm (1545 mg/kg/day) CGA-354743. These changes were not dose-dependent, were within historical limits for the laboratory, and of no toxicological and biological relevance.

Males fed 5000 ppm CGA-77102 had statistically significant increases of glucose and total serum protein, as well as decreases of AST and ALT activity. Female rats fed 5000 ppm CGA-77102 had statistically increased cholesterol and phosphate and

decreased total serum bilirubin. These changes in clinical chemistry parameters were within the reference values provided by the laboratory and of no toxicological or biological significance. The study report indicates that GGT values were increased in males and females. However, examination of the individual animal data shows that GGT was measured only in the GCA-77102 treated animals. Therefore, these values cannot be compared to control measurements. It appears that there is also no reference value for GGT from this laboratory, as it is listed as 0.0 for both males and females in Appendix D.

E. URINALYSIS

No treatment-related findings were reported for any group fed CGA-354743. Males rats fed 5000 ppm CGA-77102 had increased leukocytes in the urine (220/μL compared with 80/μL in control rats). The significance of this finding is unknown.

F. OPHTHALMIC EXAMINATION

Ophthalmic examination revealed no treatment-related changes.

G. SACRIFICE AND PATHOLOGY

1. Organ weight

Statistically significant changes to rats fed CGA-354743 in organ weights were limited to increased absolute brain weight of 20,000 ppm (1545 mg/kg/day) males, absolute kidney weight of 6000 ppm (519 mg/kg/day) females, and spleen weight relative to body weight of 20,000 ppm (1685 mg/kg/day) females. These increases, though statistically significant, were unrelated to dose and not of a magnitude to be toxicologically and biologically significant.

Both male and female rats fed 5,000 ppm CGA-77102 had increased liver weights relative to control animals, although the increases were not statistically significant. Likewise, the liver weight relative to body weight of male rats was increased, though not statistically. The liver to body weight ratio of female rats was statistically significant compared with control rats.

TABLE 5. Body and liver weight and liver to body weight ratios of rats fed 5000 ppm CGA-77102

Parameter	Males		Females	
	0.0 ppm	5000 ppm	0.0 ppm	5000 ppm
Body Weight	472.9 ± 45.65	461.2 ± 52.27	258.9 ± 25.86	236.1 ± 32.10
Absolute Liver Weight	19.44 ± 3.32	21.88 ± 3.76	9.853 ± 1.09	10.14 ± 1.62
Liver to Body Weight	41.02 ± 4.92	47.35 ± 4.71	38.08 ± 2.33	42.97 ± 3.83*

*$p<0.05$
Data from pp. 161-170; MRID 44931710

2. Gross pathology

No treatment-related gross pathology findings were reported.

3. Microscopic pathology

No microscopic pathology findings were reported for rats fed CGA-354743. Four of ten female rats fed 5000 ppm CGA-77102 had minimal to slight hepatic centrilobular hypertrophy as compared to the none in the control group.

III. DISCUSSION

A. STUDY AUTHOR'S CONCLUSIONS

The study author concluded that CGA-354743 was well-tolerated up to the limit dose of 20000 ppm. The dose of 6000 ppm CGA-354743 was the NOEL and the dose of 20000 ppm is the NOAEL. (The basis for setting the NOEL/NOAEL was not given.) The dietary concentration of 5000 ppm of CGA-77102 represents the Maximum Tolerated Dose.

B. DISCUSSION

Administration of CGA-354743 in the diet to male and female Crl:CD BR rats at concentrations of 0.0, 360, 1200, 6000, or 20,000 ppm (equivalent to 0.0 25.1, 86.2, 427.0 and 1545 mg/kg/day for males and 0.0, 28.4, 98.3, 519.0 and 1685 mg/kg/day for females) for 90 days resulted in few observed effects. The highest dose tested exceeded the guideline recommended limit intake of 1000 mg/kg/day. An additional 10 rats/sex/group were administered CGA-77102 in the diet at a dose of 5000 ppm (429 mg/kg/day for males and 563 mg/kg/day for females).

All animals survived to study end and no clinical signs were reported. There were no statistically significant changes in body weight, body weight gain, food consumption, food efficiency, ophthalmoscopic examination, urinalysis, or histopathology in animals treated with CGA-354743. Dietary exposure to CGA-77102 produced a statistically significant decreased body weight gain (-20%, $p \leq 0.01$) in males during week 1 only. Females exposed to CGA-77102 showed decreased body weight gain (-19%) by week 13, but these changes were not statistically significant. The food efficiency of rats fed CGA-77102 was decreased relative to their respective control animals.

Statistically significant changes in hematologic parameters were limited to marginally increased counts for white blood cells, eosinophils, and lymphocytes of females receiving 6000 ppm (519 mg/kg/day) and the MCV of male rats receiving 6000 ppm (427.0 mg/kg/day) CGA-354743. However, these changes were not dose-dependent, were within historic reference ranges, and are not considered toxicologically or biologically relevant. Sporadic statistically significant changes in a limited number of clinical chemistry parameters were also reported. These included phosphorous for males

receiving 20,000 ppm (1545 mg/kg/day) and females receiving 6000 ppm (519 mg/kg/day), and urea for males receiving 20,000 ppm (1545 mg/kg/day) CGA-354743. These changes also were not dose-dependent, were within historical limits for the laboratory, and of no toxicological and biological relevance. Although it appeared that male and female rats fed 5000 ppm CGA-77102 had marginally increased serum GGT activity, there is some confusion about the extent of the analyses for this parameter, as discussed in Study Deficiencies. Male and female rats did have increased absolute and relative liver weights. These results are consistent with a mild liver hypertrophy observed microscopically in females. No toxicologically relevant increases of organ weights were found for rats fed CGA-345743.

The data presented in this study show that the NOAEL for CGA-354743 is ≥20,000 ppm (1545 mg/kg/day for male and 1685 mg/kg/day female rats). No LOAEL could be established. The highest dose tested for both males and females exceeded the guideline limit dose. At 5000 ppm (429 mg/kg/day in males and 563 mg/kg/day in females) CGA-77102 , there evidence of decreased body weight gain and food efficiency, increased absolute and relative liver weights and an increased incidence of hepatic centrilobular hypertrophy, although the effects were mild.

B. STUDY DEFICIENCIES

The blood chemistry summary on page 134 of MRID 44931710 lists a mean GGT values of 0.1 for group 1 (control) males. However, the individual animal data on page 270 shows values of 0.000 for all animals. In fact, based on the individual animal data, the only animals which had GGT measurements were the group 6 males and females. In addition, only 5/10 females in group 6 had values listed in the individual animal data. Section 3.6 Laboratory investigations states that laboratory investigations (hematology, blood chemistry and urine analyses) were carried out on all surviving animals at the end of the treatment period. Failure to measure GGT is not included in section 2.2 Deviations from the protocol. This irregularity should be clarified but it does not alter the final conclusions of the study.

DATA EVALUATION REPORT

Metolachlor ESA (CGA-354743 TECHNICAL)

STUDY TYPE: DEVELOPMENTAL TOXICITY- RAT [870.3700 (83-3a)]
MRID 44931711

Prepared for

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Office of Pesticide Programs
U.S. Environmental Protection Agency
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Prepared by

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Disclaimer

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

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EPA Reviewer: Virginia A. Dobozy, VMD, MPH Virginia A Dobozy Date: 4/10/01
Reregistration Branch I, Health Effects Division (7509C)
EPA Work Assignment Manager: Joycelyn Stewart, PhD Joycelyn Stewart Date: 4/25/01
Toxicology Branch, Health Effects Division (7509C)

DATA EVALUATION RECORD

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

DP BARCODE: D260393 SUBMISSION CODE: S570059
P.C. CODE: 108801 (parent) TOX. CHEM. NO.: 188DD

TEST MATERIAL (PURITY): CGA-354743 Technical (98% a.i.)

SYNONYMS: none; degradate of metolachlor

CITATION: Doubovetzky, M. (1999) CGA-354743 technical: Rat oral teratogenicity. Novartis Crop Protection AG, Toxicology, 4332 Stein, Switzerland. Laboratory Study No. 981009. January 25, 1999. MRID 44931711. Unpublished.

SPONSOR: Novartis Crop Protection, Inc., 410 Swing Road, Post Office Box 18300, Greensboro, NC 27419

EXECUTIVE SUMMARY: In a developmental toxicity study (MRID 44931711), 28 presumed pregnant Wistar B: Hanlbm:WIST rats per group were administered CGA 354743 Technical (98%; Batch No. KI-5408/6) by gavage in 0.5% aqueous sodium carboxymethylcellulose in 0.1% aqueous polysorbate 80 at doses of 0, 250, 500, or 1000 mg/kg/day on gestation days (GD) 6-15, inclusive. Controls were treated with 0.5% sodium carboxymethylcellulose in 0.1% aqueous polysorbate 80 (vehicle). On GD 21, dams were sacrificed, subjected to gross necropsy, and all fetuses examined externally. Approximately one-half of each litter was processed for visceral examination and the remaining one-half was processed for skeletal examination.

All animals survived to terminal sacrifice. No clinical signs of toxicity were observed in any animal. Maternal body weights, body weight gains, and food consumption were similar between the treated and control groups throughout the study. Maternal necropsy was unremarkable.

Therefore, the maternal toxicity NOAEL is ≥1000 mg/kg/day and the maternal toxicity LOAEL was not identified.

No differences were observed between the treated and control groups for number of corpora lutea, number of implantation sites, live fetuses/dam, pre- and post-implantation losses, fetal body weights, or fetal sex ratios.

No treatment-related external, visceral, or skeletal malformations/variations were observed in any fetus from any group.

The high dose is equivalent to the limit dose for developmental toxicity studies.

Therefore, the developmental toxicity NOAEL is ≥1000 mg/kg/day and the developmental toxicity LOAEL was not identified.

This study is classified as **Acceptable/Guideline** and satisfies the requirements for a developmental toxicity study [870.3700 (§83-3a)] in rats.

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

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material: CGA-354743 Technical

Description: solid
Batch No.: KI-5408/6
Purity: 98% a.i.
Stability of compound: not stated
CAS No.: not given
Structure: not given

2. Vehicle and/or positive control

A 0.5% (w/w) aqueous solution of sodium carboxymethylcellulose (CMC, Hercules Powder Company, Pharmacopeia quality, high viscosity, Prod. 7HF) in 0.1% aqueous polysorbate 80 was used as the vehicle and negative control. No positive control was used in this study.

3. Test animals

Species: rat
Strain: Wistar B: Hanlbm:WIST
Age and weight at study initiation: minimum of 8 weeks; 170.0-216.4 g
Source: BRL, Biological Research Laboratories Ltd., Woelferstrasse 4, CH-4414 Fuellinsdorf, Switzerland
Housing: Animals were individually housed in Macrolon cages with wire mesh tops and standardized granulated soft wood bedding material.
Diet: Pelleted certified standard feed (Nafag No. 890, Tox; Nafag, Naehr- und Futtermittel AG, Gossau, Switzerland) was available *ad libitum*.
Water: Tap water was available *ad libitum*.
Environmental conditions:
Temperature: 22 ± 3°C
Humidity: 50 ± 20%
Air changes: about 16/hour
Photoperiod: 12 hr light/dark
Acclimation period: at least 7 days between delivery from animal breeder and the first day of treatment

B. PROCEDURES AND STUDY DESIGN

This study was designed to assess the developmental toxicity potential of CGA-354743 Technical when administered by gavage to rats on GD 6-15, inclusive.

1. In life dates

   Start: April 7, 1998; end: April 28, 1998 (start of necropsy)

2. Mating

   Females were mated to a male of the same stock and proven fertility at a ratio of three females to one male. Each cage was divided into two parts by a guillotine door, separating the sexes until 4 p.m. on the mating day, when the door opened automatically. Successful mating was assessed by the presence of a vaginal plug or of spermatozoa in a vaginal smear. The day of successful mating was designated as gestation day (GD) 0.

3. Animal assignment and dose selection are presented in Table 1. Animals were assigned to a control or treatment group using a method of randomization based on weight stratification.

TABLE 1. Animal assignment

Test Group	Dose Level (mg/kg/day)	Number Assigned
Control	0	28
Low Dose	250	28
Mid Dose	500	28
High Dose	1000	28

Data taken from text tables pp. 15 and 16, MRID 44931711.

4. Dose selection rationale

   Doses were selected on the basis of a range-finding study (Laboratory Study No. 981008) in which pregnant rats were administered 250, 500, or 1000 mg/kg/day. No maternal or developmental toxicity was observed at any dose. Further details of this study were not included in the report.

5. Dose solution preparation and analysis

   The test substance was mixed in a 0.5% aqueous solution of sodium carboxymethylcellulose in 0.1% aqueous polysorbate 80. Solutions were prepared daily with a high-speed homogenizer. Homogeneity during administration was maintained with a magnetic stirrer. Samples of the dosing solutions were analyzed for concentration, homogeneity, and stability three times during the study. Samples from the top, middle, and bottom of the dosing solutions were analyzed for concentration and homogeneity.

Stability was determined after storage at room temperature for the duration of dosing from samples taken from the middle of the solutions.

Results -
Concentration analysis: Absence of test article was confirmed in the vehicle. Mean concentrations of the dosing solutions ranged from 99.5% to 104% of nominal.

Homogeneity analysis: Concentrations of the top, middle, and bottom of the dosing solutions differed by <10%.

Stability analysis: Samples taken after the period of dosing differed from their initial measured concentrations by <6%.

Analyses of the dosing solutions indicated that the test article could be adequately mixed in the vehicle, was stable for the duration of use, and that actual doses to the animals were acceptable.

6. Dosing

All doses were administered in a volume of 10 mL/kg of body weight.

C. OBSERVATIONS

1. Maternal observations and evaluations

The animals were checked once daily for clinical signs and twice daily for mortality. Body weights were measured daily and food consumption was measured on days 6, 11, 16, and 21. Dams were sacrificed on GD 21 by carbon dioxide inhalation and examined grossly. The number of corpora lutea on each ovary was counted. Gravid uteri were weighed and examined for number and location of live and dead fetuses and number and location of early and late resorptions and abortion sites. Dams found dead or sacrificed early were subjected to gross necropsy.

2. Fetal evaluations

At necropsy, each live fetus was weighed, sexed, and examined for external abnormalities. Fetuses were killed by subcutaneous injection of a barbiturate anesthetic. Approximately one-half of each litter was processed for visceral examination and the remaining one-half processed for skeletal examination. In the case of a gross external anomaly or malformation, fetuses were allocated to one technique depending on the type and incidence of the finding. For the visceral examinations, fetuses were fixed in Bouin's solution for at least two weeks and then micro-dissected. For the skeletal examinations, fetuses were cleared with potassium hydroxide and stained with alizarin red S.

D. DATA ANALYSIS

1. Statistical analysis

Continuous data were analyzed by the Analysis of Variance (ANOVA) followed by Dunnett's t-test to separate the means. The Chi-Square and Fisher's Exact tests were used for the analysis of categorical data. Non-parametric data were analyzed with the Kruskal-Wallis test followed by the Mann-Whitney U test.

2. Historical control data from September 19, 1970 to December 31, 1998 on 432 mated females were provided to allow comparison with concurrent controls and treatment groups.

II. RESULTS

A. MATERNAL TOXICITY

1. Mortality and clinical signs

All animals survived to scheduled sacrifice. No treatment-related clinical signs of toxicity were observed in any animal.

2. Body weight

Selected maternal body weights during gestation are given in Table 2. No statistically significant differences in absolute body weights occurred at any time between the treated groups and the control group. Body weight gains were also similar between the treated and control groups throughout the study.

TABLE 2: Maternal body weights during gestation (g)				
GD	**0 mg/kg/day**	**250 mg/kg/day**	**500 mg/kg/day**	**1000 mg/kg/day**
0	190.6 ± 9.8	190.2 ± 10.0	189.8 ± 9.2	190.0 ± 9.2
6	212.9 ± 9.7	211.6 ± 8.6	211.3 ± 8.4	212.4 ± 9.9
10	228.4 ± 10.5	227.2 ± 9.4	227.0 ± 10.4	229.1 ± 10.6
16	262.9 ± 13.5	262.9 ± 12.6	262.4 ± 14.5	265.1 ± 14.3
21	315.6 ± 19.3	320.5 ± 18.1	317.4 ± 19.1	318.8 ± 20.7
Adjusted body wt.[a]	245.2	246.8	245.8	245.8

Data taken from Tables 2 and 7, pp. 31-33 and 46, respectively, MRID 44931711.
[a]Adjusted body weight = terminal body weight - gravid uterine weight.

3. Food consumption

Maternal food consumption was similar between the treated and control groups throughout the study.

4. Gross pathology

No treatment-related gross abnormalities were observed at maternal necropsy.

5. Cesarean section data

Data collected at cesarean section are summarized in Table 3. No differences were observed between the treated and control groups for number of corpora lutea, number of implantation sites, live fetuses/dam, resorptions, pre- and post-implantation losses, fetal body weights, or fetal sex ratios. No dam had complete litter resorption or contained dead fetuses.

TABLE 3: Cesarean section observations				
Observation	**0 mg/kg/day**	**250 mg/kg/day**	**500 mg/kg/day**	**1000 mg/kg/day**
No. Animals Assigned	28	28	28	28
No. Animals Pregnant	27	25	27	28
Pregnancy Rate (%)[a]	96.4	89.3	96.4	100
Maternal Mortality	0	0	0	0
Delivered Early/Aborted	0	0	0	0
Gravid Uterine Wt (g)	70.4	73.7	71.7	73.0
Corpora Lutea/Dam	11.1	11.3	11.1	11.5
Implantation/Dam	10.7	11.0	10.6	11.1
Preimplantaion Loss (mean %)	4.1	2.8	4.7	4.4
Postimplantaion Loss (mean %)	4.0	1.3	1.4	4.1
Total Live Fetuses	279	272	282	302
Live Fetuses/Litter	10.3	10.9	10.4	10.8
Mean Fetal Weight (g)	4.9	5.0	5.0	5.0
Sex Ratio (% Male)	48.7	45.2	46.1	48.7
Total Dead Fetuses	0	0	0	0
Dams With All Resorptions	0	0	0	0
Resorptions/Dam				
Early Resorptions	0.4	0.2	0.1	0.4
Late Resorptions	0.0	0.0	0.0	0.0

Data taken from Tables 5, 6, and 7, pp. 40, 42-44, and 46, respectively, MRID 44931711.
[a]Calculated by reviewer.

B. DEVELOPMENTAL TOXICITY

No treatment-related external, visceral, or skeletal malformations/variations were observed in any fetus from any group. A summary of findings is given in Table 4.

1. External examination

The number of fetuses(litters) examined for external malformations/variations in the 0, 250, 500, and 1000 mg/kg/day groups was 279(27), 272(25), 282(27), and 302(28), respectively. One high-dose litter contained a fetus with an umbilical hernia.

2. Visceral examination

The number of fetuses(litters) examined for visceral malformations/variations in the 0, 250, 500, and 1000 mg/kg/day groups was 130(27), 128(25), 135(27), and 143(27), respectively. One mid-dose fetus had most organs in situs inversus. Anophthalmia and hemorrhagic liver were also observed in the high-dose fetus with the umbilical hernia. Anomalies such as thymic remnant in the neck and accessory lobulets on the liver were seen in one to five fetuses per group including controls.

3. Skeletal examination

The number of fetuses(litters) examined for skeletal malformations/variations in the 0, 250, 500, and 1000 mg/kg/day groups was 149(27), 143(25), 146(27), and 159(28), respectively. The only skeletal malformation was fused ribs in one low-dose fetus. Skeletal anomalies of the sternebrae, vertebrae, and ribs were observed at low incidences in fetuses from the treated and control groups. Variations in ossification rates of the cranial bones, metatarsals, sternebrae, calcaneus, vertebrae, ribs, and phalanges were also common to fetuses from all groups.

TABLE 4: Fetal external, visceral, and skeletal observations no. fetuses (no. litters) affected				
Observation	**0 mg/kg/day**	**250 mg/kg/day**	**500 mg/kg/day**	**1000 mg/kg/day**
External				
Total external findings (umbilical hernia)	0 (0)	0 (0)	0 (0)	1 (1)
Visceral				
Umbilical hernia	0 (0)	0 (0)	0 (0)	1 (1)
Most organs in situs inversus	0 (0)	0 (0)	1 (1)	0 (0)
Anophthalmia	0 (0)	0 (0)	0 (0)	1 (1)
Hemorrhagic liver	0 (0)	0 (0)	0 (0)	1 (1)
Thymic remnant in the neck	1 (1)	1 (1)	3 (3)	1 (1)
Accessory liver lobulet	0 (0)	3 (3)	5 (5)	2 (2)
Total visceral observations	1 (1)	4 (3)	9 (7)	4 (4)
Skeletal				
Total skeletal malformations (fused ribs)	0 (0)	1 (1)	0 (0)	0 (0)
Total skeletal anomalies	9 (7)	10 (7)	17 (11)	11 (8)
Total skeletal variations	147 (27)	138 (25)	144 (27)	155 (27)

Data taken from Tables 9, 10, and 11-13, pp. 50, 52-55, and 58-96, respectively, MRID 44931711.

III. DISCUSSION

A. INVESTIGATORS' CONCLUSIONS

The study author concluded that no signs of maternal or fetal toxicity and no evidence of teratogenicity were observed following maternal treatment with CGA 354743 Technical on GD 6-15; the NOEL for rat dams and fetuses was 1000 mg/kg/day.

B. REVIEWER'S DISCUSSION

1. MATERNAL TOXICITY

Maternal toxicity was not evident in any treated group. No clinical signs were observed and body weights, body weight gains, and food consumption were similar between the treated and control groups.

Therefore, the maternal toxicity NOAEL is ≥1000 mg/kg/day and the maternal toxicity LOAEL was not identified.

2. DEVELOPMENTAL TOXICITY

a. Deaths/resorptions

Maternal treatment with the test article did not result in increases in either pre- or postimplantation loss or fetal death.

b. Altered growth

No treatment-related effects on fetal body weights or ossification rates were observed.

c. Developmental variations

Developmental variations were common to both treated and control fetuses and the incidence rates of specific variations were not affected by treatment.

d. Malformations

Malformations did not increase with exposure to the test article.

It should be noted that although neither maternal nor developmental toxicity were apparent, the high dose is equivalent to the limit dose for developmental toxicity studies.

Therefore, the developmental toxicity NOAEL is ≥1000 mg/kg/day and the developmental toxicity LOAEL was not identified.

C. STUDY DEFICIENCIES

No deficiencies were identified that would compromise the integrity of this study.

D. CORE CLASSIFICATION

This study is classified as **Acceptable/Guideline** and satisfies the requirements for a developmental toxicity study [870.3700 (§83-3a)] in rats.

DATA EVALUATION REPORT

CGA-354743

***SALMONELLA/ESCHERICHIA*/MAMMALIAN ACTIVATION GENE MUTATION ASSAY; [OPPTS 870.5100 (§84-2)]**
MRID 44931712

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
Life Sciences Division
Oak Ridge National Laboratory
Oak Ridge, TN 37831
Task Order No. 00-09I

Primary Reviewer: B.L. Whitfield, Ph.D.	Signature: Date:	B L Whitfield APR 2 5 2001
Secondary Reviewers: Cheryl B. Bast, Ph.D., D.A.B.T.	Signature: Date:	Cheryl B Bast APR 2 5 2001
Robert H. Ross, Group Leader, M.S.	Signature: Date:	Robert H. Ross APR 2 5 2001
Quality Assurance: LeeAnn Wilson, M.A.	Signature: Date:	L. A. Wilson APR 2 5 2001

Disclaimer

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

Managed by Lockheed Martin Energy Researh Corp. for the U.S. Department of Energy under Contract No. DE-AC05-96OR22464

EPA Reviewer: Virginia A. Dobozy, VMD, MPH Virginia A Dobozy, Date 7/5/01
Reregistration Branch I, Health Effects Division (7509C)
EPA Work Assignment Manager: Joycelyn Stewart, PhD Joycelyn E Stewart, Date 7/11/2001
Toxicology Branch, Health Effects Division (7509C)

DATA EVALUATION RECORD

STUDY TYPE: *Salmonella/Escherichia*/mammalian activation gene mutation assay; [OPPTS 870.5100 (§84-2)]

DP BARCODE: D260393 SUBMISSION CODE: S570059
P.C. CODE: 108801 (parent) TOX. CHEM. NO.: 188DD

TEST MATERIAL (PURITY): CGA-354743 tech. (cga-354743 (Metolachlor ESA); 95% a.i.)

SYNONYMS: none provided

CITATION: Ogorek, B. (1996) CGA-354743: Final report - *Salmonella* and *Escherichia*/mammalian-microsome mutagenicity test. CIBA-Geigy Limited, Genetic Toxicology, Basle, Switzerland. Laboratory study ID 951133, Novartis No. 813-95, January 15, 1996. MRID 44931712. Unpublished.

SPONSOR: Novartis Crop Protection, Inc., 410 Swing Road, Post Office Box 18300, Greensboro, NC 27419

EXECUTIVE SUMMARY: In a reverse gene mutation assay in bacteria (MRID 44931712), strains TA98, TA100, TA102, TA1535 and TA1537 of *S. typhimurium* and strain WP2(uvrA) of *E. coli* were exposed to CGA-354743 tech. (Batch No. RV-2816/1, 95% a.i.) in DMSO at concentrations of 312.5, 625.0, 1250.0, 2500.0 and 5000.0 µg/plate in the presence and absence of mammalian metabolic activation (S9-mix). The S9-fraction was obtained from Aroclor 1254 induced male RAI (Tif:RAIf (SPF)) rat liver.

CGA-354743 tech. was tested up to a limit concentration of 5000 µg/plate. No cytotoxicity, as measured by thinning or absence of the background lawn of bacteria or by a reduction in the number of revertants per plate compared to the solvent control values, was seen in the preliminary cytotoxicity test or in the mutagenicity tests at concentrations up to 5000 µg/plate, with or without S9-mix. An initial and a confirmatory mutagenicity assay was conducted and all plating was in triplicate. The number of revertants per plate was not increased over solvent control values in any strain at any tested concentration, with or without S9-mix, in either assay. The positive and solvent control values were appropriate in the respective strains and within the laboratory's historical control ranges. **There was no evidence of induced mutant colonies over background.**

This study is classified as **Acceptable/Guideline**. It satisfies the requirement for FIFRA Test Guideline 84-2 (OPPTS 870.5100) 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: CGA-354743 tech.

   Description: solid
   Lot/Batch #: RV-2816/1
   Purity: 95% a.i.
   Stability of compound: stable
   CAS #: not provided
   Structure: not provided
   Solvent used: DMSO
   Other comments: metabolite of CGA-24705, metolachlor

2. Control materials

   Negative:
   Solvent/final concentration: DMSO / 0.1 mL/plate

   Positive:
   Nonactivation:
   Sodium azide 2.0 µg/plate TA100, TA1535
   2-Nitrofluorene 5.0 µg/plate TA98.
   9-Aminoacridine 80.0 µg/plate TA1537
   Mitomycin C 0.5 µg/plate TA102
   4-Nitroquinoline (4NQO) 2.0 µg/plate WP2(uvrA)

   Activation:
   2-Aminoanthracene 1.5 µg/plate TA98, TA100, TA1537
   2-Aminoanthracene 5.0 µg/plate TA102
   2-Aminoanthracene 20.0 µg/plate WP2(uvrA)
   Cyclophosphamide 200.0 µg/plate TA1535

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: CGA-354743 tech.

   Description: solid
   Lot/Batch #: RV-2816/1
   Purity: 95% a.i.
   Stability of compound: stable
   CAS #: not provided
   Structure:
   Solvent used: DMSO
   Other comments: metabolite of CGA-24705

2. Control materials

   Negative:
   Solvent/final concentration: DMSO / 0.1 mL/plate

   Positive:
   Nonactivation:
   Sodium azide 2.0 µg/plate TA100, TA1535
   2-Nitrofluorene 5.0 µg/plate TA98.
   9-Aminoacridine 80.0 µg/plate TA1537
   Mitomycin C 0.5 µg/plate TA102
   4-Nitroquinoline (4NQO) 2.0 µg/plate WP2(uvrA)

   Activation:
   2-Aminoanthracene 1.5 µg/plate TA98, TA100, TA1537
   2-Aminoanthracene 5.0 µg/plate TA102
   2-Aminoanthracene 20.0 µg/plate WP2(uvrA)
   Cyclophosphamide 200.0 µg/plate TA1535

3. Activation: S9 derived from male RAI (Tif:RAIf (SPF)) rats.

x Aroclor 1254	x induced	x rat	x liver
__ phenobarbital	__ non-induced	__ mouse	__ lung
__ none	__ other	__ other	

S9 mix composition (if purchased, give details):

S9-fraction	100.0 µL/mL
NADP	4.0 µmol/mL
$MgCl_2$	8.0 µmol/mL
KCl	33.0 µmol/mL
Na-phosphate buffer (pH 7.4)100.	0 µmol/mL
Glucose-6-phosphate	5.0 µmol/mL

4. Test organisms: *S. typhimurium* strains

__ TA97 _x_ TA98 _x_ TA100 _x_ TA102 __ TA104
_x_ TA1535 _x_ TA1537 __ TA1538; list any others:

E. coli strain WP2(uvrA)

Properly maintained? Y
Checked for appropriate genetic markers (rfa mutation, R factor)? Y

5. Test compound concentrations used

Preliminary cytotoxicity test: TA100 and WP2(uvrA), single plating
Nonactivated and activated conditions: 20.58, 61.73, 185.19, 555.56, 1666.67, 5000.00 µg/plate

Mutagenicity assay (initial and confirmatory): all strains, triplicate plating
Nonactivated and activated conditions: 312.5, 625.0, 1250.0, 2500.0, 5000.0 µg/plate

B. TEST PERFORMANCE

1. Type of Salmonella assay:

_x_ standard plate test
_ pre-incubation (_ minutes)
_ "Prival" modification *(i.e. azo-reduction method)*
_ spot test
_ other [describe]

2. Protocol

A standard pour plate assay was conducted by mixing 0.1 mL of an overnight culture of a tester strain with 2 mL top agar (0.6% agar and 0.6% NaCl supplemented with 10% of 0.5 mM L-histidine and 0.5 mM (+)biotin for the TA strains and with 10% of 0.5 mM L-tryptophan for WP2(uvrA)), 0.5 mL of S9-mix or 0.5 mL of 100 mM

sodium phosphate buffer and 0.1 mL of test material solution, positive control or solvent control. The mixture was poured onto 20 mL of minimal agar in a Petri dish (1.5% agar supplemented with 2% salts of the Vogel-Bonner Medium E and 2% glucose). The plates were inverted and incubated for approximately 48 hours at 37 ± 1.5°C in darkness. The number of revertant colonies was then counted electronically using an Artek Colony Counter or manually if a precipitate, agar damage or strong coloration of the agar interfered with automatic counting. The background lawn of bacteria was also evaluated.

Criteria for a positive response were at least a reproducible 2-fold increase in the mean number of revertants per plate above that of the solvent control at any test material concentration in strains TA98, TA1535, TA1537 or WP2(uvrA) or at least a 1.5-fold increase in strains TA100 or TA102. In general, a positive dose-response should be seen.

II. **REPORTED RESULTS** The concentrations of test material in solution were determined by HPLC with UV detection to be in agreement with the intended concentrations.

A. PRELIMINARY CYTOTOXICITY ASSAY

Five concentrations of CGA-354743 tech. ranging from 20.58 to 5000.00 µg/plate were tested, with and without S9-mix, using strains TA100 and WP2(uvrA). No cytotoxicity, as determined by a decrease in the number of revertants per plate compared to the solvent control or by a thinning or absence of the background lawn, was seen at any concentration of test material, with or without S9-mix, in either strain. The limit dose of 5000 µg/plate was thus selected as the upper dose for the mutagenicity assays.

B. MUTAGENICITY ASSAY

Five concentrations of CGA-354743 tech. ranging from 312.5 to 5000.0 µg/plate were tested, with and without S9-mix, in all six tester strains in an initial and a confirmatory assay. All plating was in triplicate. The background lawn of bacteria was normal in all strains at all test material concentrations, with or without S9-mix, in both assays. Likewise, the number of revertants per plate was not increased over solvent control values in any strain at any tested concentration, with or without S9-mix, in either assay. The positive and solvent control values were appropriate in the respective strains and within the laboratory's historical control ranges. Results of the mutagenicity assays are presented in Appendix Tables 1 - 4 (MRID 44931712, pp. 26 - 29).

III. **REVIEWER'S DISCUSSION/CONCLUSIONS**:

A. This is an acceptable study. CGA-354743 tech. was tested to a limit dose of 5000 µg/plate, suitable experimental protocol was followed and the positive and solvent control values were appropriate for the respective strains. The test material did not

increase the number of revertant colonies per plate over solvent control values in any tester strain at any evaluated concentration, with or without S9-mix.

This study is classified as **Acceptable/Guideline**. It satisfies the requirement for FIFRA Test Guideline 84-2 (OPPTS 870.5100) for *in vitro* mutagenicity [bacterial reverse gene mutation] data.

B. STUDY DEFICIENCIES

No study deficiencies were identified.

APPENDIX

MRID 44931712

THE FOLLOWING ATTACHMENTS ARE NOT AVAILABLE ELECTRONICALLY. SEE THE FILE COPY.

TABLE 1 : SUMMARY OF THE MUTAGENICITY EXPERIMENTS
Experiments with metabolic activation

Test number : 951133
Experiment : Original
Test substance : CGA 354743 tech.

Strain	Treatment	Mean Counts	Strain	Treatment	Mean Counts
TA 100	Negative control	132.33	TA 1535	Negative control	16.00
	312.50 µg/plate	134.00		312.50 µg/plate	14.67
	625.00 µg/plate	125.33		625.00 µg/plate	15.33
	1250.00 µg/plate	114.33		1250.00 µg/plate	14.00
	2500.00 µg/plate	121.67		2500.00 µg/plate	15.33
	5000.00 µg/plate	134.67		5000.00 µg/plate	12.67
	Positive control	1829.33		Positive control	213.00
WP2 uvrA	Negative control	24.67	TA 98	Negative control	37.00
	312.50 µg/plate	17.67		312.50 µg/plate	36.00
	625.00 µg/plate	18.00		625.00 µg/plate	32.67
	1250.00 µg/plate	30.00		1250.00 µg/plate	34.33
	2500.00 µg/plate	26.00		2500.00 µg/plate	32.33
	5000.00 µg/plate	29.00		5000.00 µg/plate	32.00
	Positive control	926.67		Positive control	1907.33
TA 1537	Negative control	12.33	TA 102	Negative control	287.00
	312.50 µg/plate	12.00		312.50 µg/plate	298.67
	625.00 µg/plate	14.00		625.00 µg/plate	295.67
	1250.00 µg/plate	16.67		1250.00 µg/plate	312.00
	2500.00 µg/plate	9.67		2500.00 µg/plate	286.67
	5000.00 µg/plate	13.00		5000.00 µg/plate	301.00
	Positive control	419.00		Positive control	1725.00

TABLE 2 : SUMMARY OF THE MUTAGENICITY EXPERIMENTS
Experiments without metabolic activation

Test number : 951133
Experiment : Original
Test substance : CGA 354743 tech.

Strain	Treatment	Mean Counts	Strain	Treatment	Mean Counts
TA 100	Negative control	121.00	TA 1535	Negative control	17.67
	312.50 µg/plate	116.33		312.50 µg/plate	15.33
	625.00 µg/plate	134.00		625.00 µg/plate	13.67
	1250.00 µg/plate	110.33		1250.00 µg/plate	14.33
	2500.00 µg/plate	128.00		2500.00 µg/plate	14.00
	5000.00 µg/plate	114.33		5000.00 µg/plate	14.67
	Positive control	793.33		Positive control	739.00
WP2 uvrA	Negative control	20.00	TA 98	Negative control	21.00
	312.50 µg/plate	21.00		312.50 µg/plate	18.00
	625.00 µg/plate	20.33		625.00 µg/plate	17.33
	1250.00 µg/plate	15.67		1250.00 µg/plate	16.33
	2500.00 µg/plate	19.00		2500.00 µg/plate	20.67
	5000.00 µg/plate	20.33		5000.00 µg/plate	17.33
	Positive control	1036.33		Positive control	838.00
TA 1537	Negative control	14.67	TA 102	Negative control	264.67
	312.50 µg/plate	12.33		312.50 µg/plate	276.00
	625.00 µg/plate	10.67		625.00 µg/plate	261.33
	1250.00 µg/plate	10.67		1250.00 µg/plate	287.33
	2500.00 µg/plate	11.33		2500.00 µg/plate	292.33
	5000.00 µg/plate	10.33		5000.00 µg/plate	274.33
	Positive control	771.67		Positive control	918.67

TABLE 3 : SUMMARY OF THE MUTAGENICITY EXPERIMENTS
Experiments with metabolic activation

Test number : 951133
Experiment : Confirmatory
Test substance : CGA 354743 tech.

Strain	Treatment	Mean Counts	Strain	Treatment	Mean Counts
TA 100	Negative control	101.33	TA 1535	Negative control	20.67
	312.50 µg/plate	116.67		312.50 µg/plate	18.33
	625.00 µg/plate	97.33		625.00 µg/plate	19.67
	1250.00 µg/plate	89.00		1250.00 µg/plate	17.67
	2500.00 µg/plate	105.67		2500.00 µg/plate	18.33
	5000.00 µg/plate	115.33		5000.00 µg/plate	13.67
	Positive control	1745.33		Positive control	184.67
WP2 uvrA	Negative control	30.67	TA 98	Negative control	45.67
	312.50 µg/plate	34.33		312.50 µg/plate	43.67
	625.00 µg/plate	32.00		625.00 µg/plate	42.33
	1250.00 µg/plate	31.00		1250.00 µg/plate	48.00
	2500.00 µg/plate	25.00		2500.00 µg/plate	58.00
	5000.00 µg/plate	31.33		5000.00 µg/plate	53.00
	Positive control	659.33		Positive control	1773.33
TA 1537	Negative control	12.33	TA 102	Negative control	322.67
	312.50 µg/plate	22.33		312.50 µg/plate	317.67
	625.00 µg/plate	17.67		625.00 µg/plate	300.00
	1250.00 µg/plate	17.33		1250.00 µg/plate	302.67
	2500.00 µg/plate	16.33		2500.00 µg/plate	289.67
	5000.00 µg/plate	14.00		5000.00 µg/plate	295.00
	Positive control	356.00		Positive control	1169.33

TABLE 4 : SUMMARY OF THE MUTAGENICITY EXPERIMENTS
Experiments without metabolic activation

Test number : 951133
Experiment : Confirmatory
Test substance : CGA 354743 tech.

Strain	Treatment	Mean Counts	Strain	Treatment	Mean Counts
TA 100	Negative control	109.00	TA 1535	Negative control	16.67
	312.50 µg/plate	112.33		312.50 µg/plate	19.00
	625.00 µg/plate	115.33		625.00 µg/plate	18.33
	1250.00 µg/plate	109.67		1250.00 µg/plate	21.67
	2500.00 µg/plate	104.33		2500.00 µg/plate	20.00
	5000.00 µg/plate	95.33		5000.00 µg/plate	16.33
	Positive control	934.00		Positive control	670.00
WP2 uvrA	Negative control	31.67	TA 98	Negative control	34.67
	312.50 µg/plate	30.33		312.50 µg/plate	45.00
	625.00 µg/plate	27.33		625.00 µg/plate	40.67
	1250.00 µg/plate	25.67		1250.00 µg/plate	38.00
	2500.00 µg/plate	26.33		2500.00 µg/plate	40.00
	5000.00 µg/plate	25.00		5000.00 µg/plate	36.33
	Positive control	996.67		Positive control	854.00
TA 1537	Negative control	14.00	TA 102	Negative control	336.33
	312.50 µg/plate	13.67		312.50 µg/plate	338.33
	625.00 µg/plate	14.00		625.00 µg/plate	328.67
	1250.00 µg/plate	10.67		1250.00 µg/plate	321.00
	2500.00 µg/plate	13.00		2500.00 µg/plate	337.00
	5000.00 µg/plate	13.00		5000.00 µg/plate	302.67
	Positive control	823.33		Positive control	1363.67

DATA EVALUATION REPORT

CGA-354743

STUDY TYPE: *IN VIVO* MAMMALIAN CYTOGENETICS - MICRONUCLEUS ASSAY IN MOUSE BONE MARROW CELLS [OPPTS 870.5395 (§84-2)]
MRID 44931713

Prepared for

Health Effects Division
Office of Pesticides 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. 00-09J

Primary Reviewer: B.L. Whitfield, Ph.D.	Signature: Date:	B L Whitfield FEB 0 1 2000
Secondary Reviewers: Cheryl B. Bast, Ph.D., D.A.B.T.	Signature: Date:	Cheryl B Bast FEB 0 1 2000
Robert H. Ross, Group Leader, M.S.	Signature: Date:	Robert H Ross FEB 0 1 2000
Quality Assurance: Lee Ann Wilson, M.A.	Signature: Date:	L. A. Wilson FEB 0 1 2000

Disclaimer

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

Oak Ridge National Laboratory, managed by Lockheed Martin Energy Research Corp. for the U.S. Department of Energy under contract number DE-AC05-96OR22464.

EPA Reviewer: Virginia A. Dobozy, VMD, MPH Virginia A Dobozy, Date 4/18/01
Reregistration Branch I, Health Effects Division (7509C)
EPA Work Assignment Manager: Joycelyn Stewart, PhD Joycelyn Stewart, Date 4/25/01
Toxicology Branch, Health Effects Division (7509C)

DATA EVALUATION RECORD

STUDY TYPE: *In vivo* mammalian cytogenetics - micronucleus assay in mouse bone marrow [OPPTS 870.5395 (§84-2)].

DP BARCODE: D260393 SUBMISSION CODE: S570059
P.C. CODE: 108801 (parent) TOX. CHEM. NO.: 188DD

TEST MATERIAL (PURITY): CGA-354743 tech. (CGA-354743 (Metolachlor ESA, degradate of metolachlor), 98 ± 2% a.i.)

SYNONYMS: none provided

CITATION: Deparade, E. (1998) CGA-354743: Final Report - Micronucleus test, mouse. Novartis Crop Protection AG, Toxicology, Genetic Toxicology, Basle, Switzerland. Laboratory Study ID 981016, Novartis No. 1190-98, October 19, 1998. MRID 44931713. Unpublished.

SPONSOR: Novartis Crop Protection, Inc., 410 Swing Road, Post Office Box 18300, Greensboro, NC 27419

EXECUTIVE SUMMARY: In a ICO:CD1 (CRL) mouse bone marrow micronucleus assay (MRID 44931713), five mice/sex/dose were treated once each via oral gavage with CGA-354743 tech. (Batch No. KI5408/6, 98 ± 2% a.i.) at doses of 1250, 2500 and 5000 mg/kg body weight. Bone marrow cells were harvested at 16, 24 and 48 post-treatment from the high dose and negative control groups and at 24 hours only from the intermediate and low dose and positive control groups. The vehicle was bidistilled water.

There were no signs of toxicity in the preliminary toxicity assay (5000 mg/kg only) or at any dose or sampling time in the micronucleus assay. The upper dose was the limit dose for this assay and also the solubility limit. No bone marrow cytotoxicity, based on the PCE/NCE ratio was evident. There was no statistically significant increase in the percentage of micronucleated PCEs in any CGA-354743 tech. treated group when compared to the solvent control groups and there were no sex-related differences seen. The mean percentage of micronucleated PCEs (pooled male/female data) was 0.04% in all treatment groups except the 5000 mg/kg, 24 hour harvest time group in which the percentage was 0.07%. The solvent control values were 0.03, 0.03 and 0.01% at 16, 24 and 48 hours, respectively while the positive control value was 1.07% (24 hour harvest time only). The positive and solvent controls induced the appropriate responses.

There was no significant increase in the frequency of micronucleated polychromatic erythrocytes in bone marrow after any dose or treatment time.

This study is classified as **Acceptable/Guideline**. It satisfies the requirement for FIFRA Test Guideline [OPPTS 870.5395 (§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: CGA-354743 tech.

   Description *(e.g. technical, nature, color):* solid
   Lot/Batch #: KI-5408/6
   Purity: 98 ± 2 % a.i.
   Stability of compound: stable
   CAS #: not provided
   Structure: not provided
   Solvent used: bidistilled water
   Other comments: metabolite of CGA-24705, metolachlor

2. Control materials

   Negative *(if not vehicle)*/Route of administration: none

   Vehicle/Final volume/Route of administration: bidistilled water / 10 mL/kg / oral gavage

   Positive/Final dose(s)/Route of administration: cyclophosphamide / 64 mg/kg / oral gavage

3. Test compound administration

   Volume of test substance administered: 10 mL/kg body weight

   Route of administration: oral gavage

   Dose levels used:

   Preliminary toxicity test: 5000 mg/kg

   Micronucleus assay: 1250, 2500, 5000 mg/kg

4. Test animals

Species: mouse
Strain: ICO:CD1(CRL)
Age: 6 - 8 weeks
Weight male: 32 - 38 g female: 24 - 30 g
Source: Animal farm of IFFA CREDO, France, 79592 L'Arbresle
No. animals used per dose: 5 males 5 females
Properly maintained? Y

B. TEST PERFORMANCE

1. Treatment and sampling times

a. Test compound

Dosing: x once ___ twice (24 hr apart)
___ other (describe):
Sampling (after last dose): ___ 6 hr ___ 12 hr x 24 hr x 48 hr ___ 72 hr x
(other describe): 16 hr (sampling time for the intermediate and low doses was 24 hours only)

b. Negative and/or vehicle control

Dosing: x once ___ twice (24 hr apart)
Sampling (after last dose): ___ 6 hr ___ 12 hr
x 24 hr x 48 hr ___ 72 hr (mark all that are appropriate),
other (describe): 16 hours

c. Positive control

Dosing: x once ___ twice (24 hr apart)
___ other (describe):
Sampling (after last dose): ___ 6 hr ___ 12 hr
x 24 hr ___ 48 hr ___ 72 hr (mark all that are appropriate), other (describe):

2. Tissues and cells examined

x bone marrow ___ other (list):

No. of polychromatic erythrocytes (PCE) examined per animal: 2000
No. of normochromatic erythrocytes (NCE; more mature RBCs) examined per animal: varied, the PCE/NCE ratio was determined in at least 1000 erythrocytes
Other (if other cell types examined, describe):

3. Details of slide preparation

Mice were killed at the selected harvest time by CO_2 asphyxiation, bone marrow was collected in fetal calf serum from both femurs of each animal, the marrow suspension was centrifuged and the cells resuspended in fetal calf serum. Smears were prepared and stained with May-Grünwald/Giemsa solution and mounted. No further details of slide preparation was provided. The slides were coded prior to analysis.

4. Statistical methods

The significance of differences was assessed by the Chi-Squared-Contingency-Test ($F=1$, $p < 0.05$)

5. Evaluation criteria

Micronuclei were identified as uniform, darkly stained, more or less round bodies in the cytoplasm of erythrocytes. The unit of measure was the micronucleated PCE, not the number of micronuclei. Bodies which were reflective, improperly shaped or stained or which were not in the focal plain of the cell were judged to be artifacts. The mean number of micronucleated PCEs in the treatment groups and negative control groups were compared for significant differences, using data from each sex separately and also using pooled data from both sexes.

The results were considered positive if the mean number of micronucleated PCEs in any test material treated group exceeded 0.20% and if there was a statistically significant difference (Chi Squared ≥ 3.84; $p < 0.05$) when compared with the negative control. A positive response in a minority of mice accompanied by an increase in the number of micronucleated NCEs in not considered treatment related.

II. REPORTED RESULTS

A. PRELIMINARY TOXICITY ASSAY

One male and one female mouse each received a single treatment of 5000 mg/kg CGA-354743 tech. via oral gavage and were observed for three days. No deaths or other signs of toxicity were seen. The experiment was repeated with the same results. This concentration was thus chosen as the upper dose for the micronucleus assay.

B. MICRONUCLEUS ASSAY

Five mice/sex/dose were treated once each via oral gavage with 1250, 2500 or 5000 mg/kg CGA-354743 tech. and the bone marrow cells harvested at 24 hours post-treatment in all dose groups and additionally at 16 and 48 hours post-treatment in the 5000 mg/kg groups. No signs of toxicity were seen in any mouse during the study

and no indication of bone marrow cytotoxicity was seen (based on the PCE/NCE ratios). Test material concentrations were analyzed by HPLC to confirm the actual concentrations and stability and found acceptable.

There was no statistically significant increase in the percentage of micronucleated PCEs in any CGA-354743 tech. treated group when compared to the solvent control groups and there were no sex-related differences seen. The mean percentage of micronucleated PCEs (pooled male/female data) was 0.04% in all treatment groups except the 5000 mg/kg, 24 hour harvest time group in which the percentage was 0.07%. The solvent control values were 0.03, 0.03 and 0.01% at 16, 24 and 48 hours, respectively while the positive control value was 1.07% (24 hour harvest time only). Results of the micronucleus assay are summarized in Appendix Tables 1 - 3 (MRID 44931713, pp. 22 - 24).

III. **REVIEWER'S DISCUSSION/CONCLUSIONS**

A. This is an acceptable study. CGA-354743 tech. was tested to a limit dose of 5000 mg/kg, suitable experimental protocol was followed and the positive and solvent control values were appropriate. CGA-354743 tech. did not increase the percentage of micronucleated PCEs as tested in this study.

This study is classified as **Acceptable/Guideline**. It satisfies the requirement for FIFRA Test Guideline [OPPTS 870.5395(§84-2)] for *in vivo* cytogenetic mutagenicity data.

B. STUDY DEFICIENCIES

No study deficiencies were identified.

APPENDIX

MRID 44931713

THE FOLLOWING ATTACHMENTS ARE NOT AVAILABLE ELECTRONICALLY. SEE THE FILE COPY.

TABLE 1 : MICRONUCLEUS TEST ON MOUSE BONE MARROW CELLS
SUMMARIZED DATA

ANIMALS SACRIFICED 16 h AFTER APPLICATION

Test number : 981016
Test substance : CGA 354743 tech.
Batch : KI-5408/6

Treatment Sex	PCEs counted (total)	Ratio PCE/ NCE	Micronucleated PCEs # found	% of micro-nucleated PCEs
Negative Control: Bidistilled water				
Males	10000	0.89	2	0.02
Females	10000	0.94	3	0.03
Pooled data	20000	0.92	5	0.03
Treatment: 5000 mg/kg				
Males	10000	0.79	3	0.03
Females	10000	0.86	5	0.05
Pooled data	20000	0.83	8	0.04

In a total of 5 animals per sex

TABLE 2 : MICRONUCLEUS TEST ON MOUSE BONE MARROW CELLS SUMMARIZED DATA

ANIMALS SACRIFICED 24 h AFTER APPLICATION

Test number : 981016
Test substance : CGA 354743 tech.
Batch : KI-5408/6

Treatment Sex	PCEs counted (total)	Ratio PCE/ NCE	Micronucleated PCEs # found	% of micro-nucleated PCEs
Negative Control: Bidistilled water				
Males	10000	0.76	3	0.03
Females	10000	0.75	2	0.02
Pooled data	20000	0.76	5	0.03
Treatment: 1250 mg/kg				
Males	10000	0.99	2	0.02
Females	10000	0.93	6	0.06
Pooled data	20000	0.96	8	0.04
Treatment: 2500 mg/kg				
Males	10000	0.69	3	0.03
Females	10000	0.81	4	0.04
Pooled data	20000	0.75	7	0.04
Treatment: 5000 mg/kg				
Males	10000	0.65	6	0.06
Females	10000	0.66	7	0.07
Pooled data	20000	0.66	13	0.07
Positive Control: Cyclophosphamide 64 mg/kg, p.o.				
Males	10000	0.67	109	1.09*
Females	10000	0.64	104	1.04*
Pooled data	20000	0.66	213	1.07*

In a total of 5 animals per sex
* Number of micronucleated PCEs statistically significant different from negative control (Level of significance $p \leq 0.05$)

TABLE 3 : MICRONUCLEUS TEST ON MOUSE BONE MARROW CELLS SUMMARIZED DATA

ANIMALS SACRIFICED 48 h AFTER APPLICATION

Test number : 981016
Test substance : CGA 354743 tech.
Batch : KI-5408/6

Treatment Sex	PCEs counted (total)	Ratio PCE/ NCE	Micronucleated PCEs # found	% of micro-nucleated PCEs
Negative Control: Bidistilled water				
Males	10000	0.87	1	0.01
Females	10000	0.93	1	0.01
Pooled data	20000	0.90	2	0.01
Treatment: 5000 mg/kg				
Males	10000	0.87	3	0.03
Females	10000	1.07	4	0.04
Pooled data	20000	0.97	7	0.04

In a total of 5 animals per sex

DATA EVALUATION REPORT

CGA-354743

STUDY TYPE: OTHER GENOTOXICITY: UNSCHEDULED DNA SYNTHESIS IN PRIMARY RAT HEPATOCYTES/MAMMALIAN CELL CULTURES [OPPTS 870.5550 (§84-2)]
MRID 44931714

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. 00-09K

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Disclaimer

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

Oak Ridge National Laboratory, managed by Lockheed Martin Energy Research Corp. for the U.S. Department of Energy under contract number DE-AC05-96OR22464.

EPA Reviewer: Virginia A. Dobozy, VMD, MPH Virginia A Dobozy, Date 4/18/01
Reregistration Branch I, Health Effects Division (7509C)
EPA Work Assignment Manager: Joycelyn Stewart, PhD Joycelyn Ellibert, Date 4/25/01
Toxicology Branch, Health Effects Division (7509C)

DATA EVALUATION RECORD

STUDY TYPE: Other Genotoxicity: Unscheduled DNA Synthesis in Primary Rat Hepatocytes/Mammalian Cell Cultures [OPPTS 870.5550 (§84-2)]

DP BARCODE: D260393 SUBMISSION CODE: S570059
P.C. CODE: 108801 (parent) TOX. CHEM. NO.: 188DD

TEST MATERIAL (PURITY): CGA-354743 tech. (CGA-354743 (Metolachlor ESA, degradate of metolachlor), 98% a.i.)

SYNONYMS: none given

CITATION: Ogorek, B. (1998) CGA-354743: Final Report - Autoradiographic DNA repair test on rat hepatocytes (OECD Conform) *in vitro*. Novartis Crop Protection AG, Toxicology, Genetic Toxicology, Basle, Switzerland. Laboratory Study ID 981017, Novartis No. 1189-98, November 23, 1998. MRID 44931714. Unpublished.

SPONSOR: Novartis Crop Protection, Greensboro, NC

EXECUTIVE SUMMARY: In an unscheduled DNA synthesis (UDS) assay (MRID 44931714), primary rat hepatocyte cultures were exposed to CGA-354743 tech. (Batch No. KI-5408/6, 98% a.i.) in bidistilled water at concentrations of 9.77, 39.06, 156.25, 625.00, 2500.00, 5000.00 µg/mL for 16 to 18 hours in an initial assay and to concentrations of 78.13, 156.25, 312.50, 625.00, 1250.00, 2500 µg/mL for 16 to 18 hours in a confirmatory assay. Primary hepatocytes were obtained from healthy male HANIbm:WIST(SPF) rats.

CGA-354743 tech. was tested up to cytotoxic concentrations based on cell morphology changes and reduced cell viability. A cytotoxicity test at concentrations ranging from 4.88 to 5000.00 µg/mL showed a general trend of decreasing cell viability with increasing test material concentration, decreasing from 81% viable cells at 4.88 µg/mL to 57% at 5000.00 µg/mL. Viability of the solvent controls was 88%. Effects on cell morphology were seen at concentrations of 39.06 and higher, although, sufficient cells with good morphology were available for UDS determination at all test material concentrations. One hundred and fifty cells (50/slide) were scored for UDS per treatment group. There was no increase in the net nuclear grain count, the net grain count of cells in repair or in the percentage of cells in repair over solvent control values at any concentration in either assay. The mean net nuclear grain counts remained below 1.0 at all

concentrations in both assays. The mean net nuclear grain counts of the solvent controls were 0.5 and -0.4 in the initial and confirmatory assays, respectively, while those of the positive controls were 12.3 and 9.3 in the initial and confirmatory assays, respectively. The positive and solvent controls induced the appropriate response. **There was no evidence that unscheduled DNA synthesis, as determined by radioactive tracer procedures (nuclear silver grain counts), was induced.**

This study is classified as **Acceptable/Guideline**. It satisfies the requirement for FIFRA Test Guideline (OPPTS 870.5550; 84-2) for other genotoxic mutagenicity data.

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

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: CGA-354743 tech.

   Description (e.g. technical, nature, color, stability): solid
   Lot/Batch #: KI-5408/6
   Purity: 98% a.i.
   Stability of compound: stable
   CAS #: not provided
   Structure: not provided
   Solvent used: bidistilled water
   Other comments: none

2. Control materials

   Negative: none
   Solvent/final concentration: bidistilled water
   Positive (concentrations/solvent): 2-acetylaminofluorene / 10 µg/mL / unspecified

3. Test compound concentrations used:

Preliminary cytotoxicity assay:	4.88, 9.77, 19.53, 39.06, 78.13, 156.25, 312.50, 625.00, 1250.00, 2500.00, 5000.00 µg/mL
UDS assay (first):	9.77, 39.06, 156.25, 625.00, 2500.00, 5000.00 µg/mL
UDS assay (confirmatory):	78.13, 156.25, 312.50, 625.00, 1250.00, 2500 µg/mL

4. Media

   See Section A. 6

5. Test cells

Mammalian cells in culture/primary rat hepatocytes. Primary hepatocytes from healthy male HANIbm:WIST(SPF) rats obtained from BRL/CPB, Biological Research Laboratories Ltd., Füllinsdorf, Switzerland.

6. Cell preparation:

a. Perfusion technique

The liver was perfused *in situ* through the portal vein for 8 - 10 minutes with calcium-free Hanks' solution (BSS) supplemented with EGTA (0.5 mMol/L) and $NaHCO_3$ (the perfusate was aerated with carbogen (95% O_2, 5% CO_2) to adjust the pH to about 7.3). The temperature was maintained at approximately 37°C. Perfusion was then continued for an additional 10 minutes with BSS supplemented with 0.05% collagenase, 2 mMol/L $CaCl_2$ and $NaHCO_3$ (aerated as before). The liver was then excised, placed in a dish containing BSS supplemented with 100 U/mL penicillin, 100 µg/mL streptomycin and 2.5 µg/mL amphotericin, 2 mMol/L $CaCl_2$, 0.4 mMol/L $MgSO_4$, 0.05% bovine serum albumin (BSA) and $NaHCO_3$. The pH was adjusted to 7.3 as before.

b. Hepatocyte harvest/culture preparation

The Glisson's capsule was opened and the cells dispersed by gently shaking the liver in the solution. Cells were filtered, washed once and resuspended in Williams' medium E. Viability of the cells, as determined by Trypan blue exclusion, was typically greater than 80%. The isolated hepatocytes in Williams Medium E containing 10% fetal bovine serum (FBS), 100 U/mL penicillin, 100 µg/mL streptomycin, 2.5 µg/mL amphotericin and 2 mMol/L glutamine were cultured in a humidified atmosphere with 5% CO_2 at 37°C. Cultures were prepared for the UDS assay by seeding 10^5 cells/mL in a series of compartments in multiplates containing gelatinized THERMANOX coverslips (4 x 10^5 cells per compartment). Following a 1.5 to 2.0 hour attachment period, unattached cells were removed by washing with BSS and the cultures were refed with culture medium.

B. TEST PERFORMANCE

1. Cytotoxicity assay

Eleven concentrations of CGA-354743 tech. ranging from 4.88 to 5000 µg/mL were tested for cytotoxicity to the hepatocytes. Two cultures per test material concentration (or solvent control) were treated with 200 µL each of the desired test solution and incubated for 16 - 18 hours. The medium was then removed and the cells washed twice with BSS and stained with Trypan blue solution (0.2%) for five minutes. The

cells were then washed with BSS, fixed and the percentage of viable cells (those unstained) in 100 cells was determined. The morphological quality of the viable cells was also evaluated.

2. UDS assay

a. Treatment

Four cultures per treatment group (solvent control, positive control and test material) were treated by adding 20 µL of test solution to 2 mL of medium in each culture compartment. Immediately after the test material was added, 8 µCi ^{3}H-thymidine was added to each compartment and the cultures incubated for 16-18 hours. Following treatment, the cells were washed twice with BSS and the nuclei swollen by treatment with 1% sodium citrate for 10 minutes. The cells were then fixed with ethanol:acetic acid (3:1 (v:v)) and the coverslips mounted on microscope slides and prepared for autoradiography.

b. Preparation of Autoradiographs/Grain Development

Slides were coated with Ilford K.5 emulsion (diluted with two volumes of water) in a dark room at 20°C, air-dried and exposed in light- and airproof boxes containing desiccants at 4°C for four days. The autoradiographs were developed in Kodak Developer D-19, rinsed in acetic acid (1%) and fixed in Hypam solution (Ilford, diluted 1:10 in water). They were then stained in hematoxylin solution, rinsed in tap water and counterstained in eosine. Slides were coded prior to scoring.

c. Grain counting

Three slides (50 cells/slide) were scored from each treatment group and control group using an electronic counter attached to a microscope at 2000x magnification. The number of silver grains over the nuclei (nuclear grain count) were counted and the mean and standard deviations were calculated. The number of silver grains over three nuclear sized regions of cytoplasm adjacent to the nucleus were also counted and the mean value subtracted from the nuclear grain count to obtain the net nuclear grain count. The percentage of cells in repair, defined as cells with a net nuclear grain count of 2.0 or more, was also determined. Cells undergoing replicative DNA synthesis were excluded from the analysis.

e. Evaluation criteria

Criteria for a positive response were a reproducible increase, compared to the solvent control, of the mean nuclear grain counts and the mean net nuclear grain counts at two or more consecutive concentrations with at least one concentration giving a mean net nuclear grain count of 2.0 or higher. In addition, the results

were considered positive if the percentage of cells in repair showed an obvious shift to higher values at two or more consecutive concentrations compared to the solvent controls. In general, a positive dose-response should be seen.

Results were considered negative if it was reproducibly shown that the mean nuclear grain counts and the mean net nuclear grain counts as well as the percentage of cells in repair were not significantly different from the solvent control values at any concentration and no concentration dependency was seen.

f. Statistical analysis

No statistical analysis was performed.

II. REPORTED RESULTS

The concentrations and stability of test material used in this study were confirmed by HPLC analysis.

A. Preliminary cytotoxicity assay

Cell viability and morphology were evaluated at eleven CGA-354743 tech. concentrations ranging from 4.88 to 5000.00 µg/mL. There was a general trend of decreasing cell viability with increasing test material concentration, decreasing from 81% viable cells at 4.88 µg/mL to 57% at 5000.00 µg/mL. Viability of the solvent controls was 88%. Effects on cell morphology were seen at concentrations of 39.06 and higher, although, sufficient cells with good morphology were available for UDS determination at all test material concentrations.

B. UDS assay

Six concentrations of CGA-354743 tech. ranging from 9.77 to 5000.00 µg/mL were evaluated for UDS inducing activity in an initial assay and six concentrations ranging from 78.13 to 2500.00 µg/mL were evaluated in a confirmatory assay. One hundred and fifty cells (50/slide) were scored per treatment group. There was no increase in the net nuclear grain count, the net grain count of cells in repair or in the percentage of cells in repair over solvent control values at any concentration in either assay. The mean net nuclear grain counts remained below 1.0 at all concentrations in both assays. The mean net nuclear grain counts of the solvent controls were 0.5 and -0.4 in the initial and confirmatory assays, respectively, while those of the positive controls were 12.3 and 9.3 in the initial and confirmatory assays, respectively. Results of the UDS assays are summarized in Appendix Tables 1 and 2 (MRID 44931714, pp. 24 and 27).

III. REVIEWER'S DISCUSSION/CONCLUSIONS

A. This is an acceptable study. CGA-354743 tech. was tested to a sufficiently high concentration, limited by cytotoxicity, proper experimental protocol was followed and the positive and solvent control values were appropriate (within the testing laboratory's historical control ranges). There was no evidence that CGA-354743 tech. induced UDS in primary rat hepatocytes as tested in this study.

This study is classified as **Acceptable/Guideline**. It satisfies the requirement for FIFRA Test Guideline (OPPTS 870.5550; 84-2) for other genotoxic mutagenicity data.

B. STUDY DEFICIENCIES - No study deficiencies were identified.

APPENDIX

MRID 44931714

THE FOLLOWING ATTACHMENTS ARE NOT AVAILABLE ELECTRONICALLY. SEE THE FILE COPY.

TABLE 1 Group mean net grain count values, original experiment

Dose (µg/ml)	Net nuclear grain count (NG)		Net grain count of cells in repair		Percent of cells in repair (NG ≤ 2)	
	mean	SD	mean	SD	mean	SD
Bidistilled water	0.5	0.4	2.7	0.0	20.0	7.2
10.00 2-AAF	12.3	0.5	12.3	0.5	100.0	0.0
5000.00	0.4	0.3	2.9	0.9	13.3	6.4
2500.00	0.4	0.3	2.9	0.1	13.3	4.2
625.00	0.6	0.5	3.0	0.4	18.0	4.0
156.25	0.1	0.2	2.9	0.2	17.3	4.2
39.06	-0.0	0.3	2.5	0.2	15.3	3.1
9.77	0.6	0.2	2.7	0.4	23.3	6.1

TABLE 2 Group mean net grain count values, confirmatory experiment

Dose (µg/ml)	Net nuclear grain count (NG)		Net grain count of cells in repair		Percent of cells in repair (NG ≤ 2)	
	mean	SD	mean	SD	mean	SD
Bidistilled water	-0.4	1.0	2.6	0.4	9.3	4.2
10.00 2-AAF	9.3	1.2	9.3	1.2	100.0	0.0
2500.00	0.8	0.3	3.0	0.2	20.7	11.0
1250.00	0.7	0.5	3.1	0.2	24.0	12.5
625.00	0.6	0.1	3.0	0.3	20.7	3.1
312.50	0.9	0.2	2.9	0.1	26.7	4.2
156.25	0.4	0.3	2.8	0.2	20.0	6.0
78.13	-0.3	0.2	2.9	0.3	8.0	4.0

DATA EVALUATION REPORT

CGA-354743
(METOLACHLOR ESA, degradate of Metolachlor)

STUDY TYPE: METABOLISM AND PHARMACOKINETICS – RAT
[OPPTS: 870-7485 (§85-1)]
MRID 44931715

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. 00-09L

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Disclaimer

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

Managed by Lockheed Martin Energy Research Corp., for the U.S. Department of Energy under Contract No. DE-AC05-96OR22464.

EPA Reviewer: Virginia A. Dobozy, VMD, MPH Virginia A Dobozy, Date 4/11/01
Reregistration Branch I, Health Effects Division (7509C)
EPA Work Assignment Manager: Joycelyn Stewart, PhD Joycelyn Stewart, Date 4/26/01
Toxicology Branch, Health Effects Division (7509C)

DATA EVALUATION RECORD

STUDY TYPE: Metabolism - Rat [OPPTS 870.7485 (§85-1)]

DP BARCODE: D260393
P.C. CODE: 108801 (parent)

SUBMISSION CODE: S570059
TOX. CHEM. NO.: 188DD

TEST MATERIAL (PURITY): CGA 77102 (purity 99.8%); [phenyl-U-^{14}C] CGA 77102 (purity >98.96%)

SYNONYMS: (S)-2- Chloro-N-(2-ethyl-6-methyl-phenyl)-N-(2-methoxy-1-methyl-ethyl)-acetamide; (S)-2-Chloro-N-(2-ethyl-6-methylphenyl)-N-(2-methoxy-1-methylethyl)-acetamide; S-Metalochlor

CITATION: Mewes, K. (1998). Determination of the soil metabolites CGA-354743, CGA-368208, and CGA-357704 in excreta of rats administered [Phenyl-U-^{14}C]CGA-77102. Novartis Crop. Protection, AG, CH-4002 Basle, Switzerland. Laboratory Study Identification 030AM07, Novartis Number 796-97. May 6, 1998. MRID 44931715. Unpublished.

SPONSOR: Novartis Crop. Protection, Inc., CH-4002, 410 Swing Road, Post Office Box 18300, Greensboro, NC 27419.

EXECUTIVE SUMMARY: In a metabolism study (MRID 44931715), groups of three male and three female rats were each given [phenyl-U-^{14}C] CGA 77102 (batch no. ILS-143.1; purity 98.9%) and non-radiolabeled CGA 77102 (batch no. AMS 757-101; purity 99.8%) to provide a total single oral dose of 0.5 or 100 mg/kg. Urinary and fecal excretion were monitored over 72 hours and major metabolites identified and quantified. The study focused on evaluating the presence of the metabolites CGA 354743, CGA 368208, and CGA 357704 in the excreta of rats.

There were no deaths or overt signs of toxicity attributed to the test material. Actual administered doses were 3-8% greater than nominal. Overall recovery of administered radioactivity was an acceptable 93.83-99.18%. Urinary excretion and carcass burden data implied that absorption was approximately 38-49% of the administered dose. Most (86.5-91.7%) of the radioactivity recovered at 72 hours post was associated with the urine and feces. The available data suggested that urinary excretion was slightly greater in female rats than male rats (42% for low- and high-dose females as compared to 30% and 32% of low- and high-dose males, respectively), and fecal elimination was expectedly less (approximately 13-15%) for females than for males. However, the small sample size (three rats per sex) precludes a definitive assessment of gender-specific differences in excretory pattern. Time-course data for absorption and excretion were not

200

provided. Based upon the residual radioactivity in the carcasses, accumulation in the tissues was less than 10% of the administered dose at 72 hours after administration.

Both urinary and fecal metabolites were identified and quantified. For both routes of elimination, three major metabolites were identified but none represented more than 0.25% of the administered dose. Characterization of these metabolites and comparison to known reference standards revealed them to be CGA 357704, CGA 354743, and CGA 368208. In the feces, CGA 357704 and CGA 354743 represented a notably greater percent of the administered dose than in the urine. The amounts of CGA 368208 were similar in the feces and urine. Biliary excretion experiments were not performed and, therefore, no assessment can be made regarding biliary or gut microflora as the source of these biotransformation products in the feces.

This metabolism study in rats is **Acceptable/Non-guideline**. Although not satisfying the requirements for a Metabolism and Pharmacokinetics study [OPPTS 870.7485 (85-1)], the study was well designed and conducted, and provided supplemental data regarding the quantitation and identification of urinary and fecal metabolites in rats given a single oral dose of CGA 77102.

COMPLIANCE: Signed and dated Good Laboratory Practice Compliance Statement (p. 3), OECD Principles of Good Laboratory Practice (p. 5), Quality Assurance (p. 6), and Data Confidentiality statements (p. 2) were provided in the study report.

I. MATERIALS AND METHODS

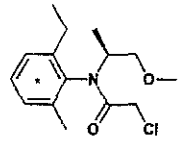
A. MATERIALS

1. Test compound

Radiolabeled: [Phenyl-U-^{14}C] CGA 77102
Batch No.: ILS-143.1
Specific Activity: 2000 kBq/mg
Purity: 98.9%
Description: not specified
Contaminants: none noted
CAS No.: 51218-45-2

Non-radiolabeled: CGA 77102
Purity: 99.8%
Batch No.: AMS 757-101
Description: not specified
Contaminants: none noted
CAS No.: 51218-45-2

Structure:



* [U-^{14}C]

2. Vehicle

Ethanol/PEG 200/water (3/2/3, v:v:v) served as the dosing vehicle.

3. Test animals

Species: rat
Strain: Tif:RA f (SPF)
Age and weight at study initiation: 7-9 weeks; males: 196-205 g, females: 182-199 g
Housing: Maintained individually in Plexiglass metabolism cages from Day-1 to termination.
Diet: Certified standard powdered diet (Nafag No. 890, NAFAG, Gossau, Switzerland, *ad libitum* (except the night before administration of radiolabeled test material).
Water: tap water *ad libitum*
Environmental conditions:
Temperature: 20°C

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Humidity: 42-78%
Air changes: not specified
Photoperiod: 12 hrs/12 hrs
Acclimation period: At least 4 days

4. Preparation of dosing solution

The dose solutions were prepared by dissolving an appropriate amount of the test material in ethanol/PEG 200/water (3/2/3, v:v:v) to provide concentrations of 0.2 mg/mL (low dose) or 24.9 mg/mL (high dose). The low- and high-doses represented approximately 212 kBq and 6.8 MBq/animal, respectively.

Results –
Homogeneity: Not specified but dosing solution preparation as described would appear to provide acceptable homogeneity.

Stability: Based upon TLC analysis (radiochromatograms provided in study report), the dosing solutions were stable at the time of administration.

Dose confirmation: The test material represented <98% of the radioactivity in the dosing solutions. Actual doses to the test animals were 3-8% greater than nominal.

B. STUDY DESIGN AND METHODS

1. Group arrangements

Animals were numbered randomly and assigned to experimental groups shown in Table 1.

TABLE 1. Study design

Experimental group	Dose (mg/kg)	Number/Sex	Remarks
Low dose (B3)	0.5	3 males 3 females	Actual administered dose ranged from 0.52 to 0.55 mg/kg; urine and fecal samples collected at 0-24, 24-48, and 48-72 hrs
High dose (D2)	100	3 males 3 females	Actual administered dose ranged from 102.30 to 111.85 mg/kg; urine and fecal samples collected at 0-24, 24-48, and 48-72 hrs

Information taken from p. 19 and Tables 1 and 2, p. 33. MRID 44931715.

2. Dosing and sample collection

Animals were administered the test material by stomach tube. Low-dose rats were given about 0.5 mL and high-dose rats were given about 0. 8 mL of the dosing solution. Individual animal doses based on individual body weights were provided in the study report.

Expired air - Not collected.

Blood - Not collected.

Urine - Urine samples were collected from individual rats at 0-24, 24-48, and 48-72 hours. Samples were collected in containers immersed in dry ice and volumes recorded. Samples were kept frozen until analyzed.

Feces - Feces were collected at ambient temperature from individual rats at 0-24, 24-48, and 48-72 hours. Sample weights were recorded and the samples were kept frozen until analyzed.

Cage wash - At the end of each sample collection period, the cages were thoroughly rinsed with water/ethanol (1/1, v:v).

Tissues - Specific tissues not collected. At termination of the experiment, the rats were anesthetized with carbon dioxide and killed by exsanguination. The carcasses were kept frozen until homogenization and analysis.

3. Sample preparation/analysis

Urine - Aliquots (0.05 - 0.5 mL) of urine samples were mixed with scintillation cocktail (Irgasafe Plus, Packard Instrument Co.) and subjected to Liquid Scintillation Counting (LSC). Approximately one half of the 0-72 hour urine samples from each dose group were pooled for the purpose of metabolite identification. Based on radioactivity content, these pooled aliquots represented 36.2% of the low dose and 37.0% of the high dose. The pooled aliquots for metabolite identification were acidified with trichloracetic acid and fractionated on a PRP-1 resin extraction column. Resin column fractionation yielded three fractions. One fraction (first methanol eluate) contained approximately 90% of the radioactivity and was used for metabolite isolation. This fraction was evaporated and analyzed by TLC with known reference standards of CGA 354743, CGA 368208, and CGA 357704. Preparative TLC was used to acquire samples of the three fractions for LC/MS/MS analysis. Urinary metabolites were quantified using solid phase extraction and HPLC.

Feces - Approximately one half of the 0-72 hour fecal samples were also pooled. These aliquots represented 53% of the low dose and 50.9% of the high dose radioactivity in the feces. For radioactivity determination, the fecal samples were homogenized with water, combusted in a sample oxidizer (Carbosorb used to trap carbon dioxide), and mixed with Permafluor E+[3] for LSC. Extraction of the fecal aliquot for metabolite identification was performed using acetonitrile and three subsequent 0.01M acetonitrile/ammonium "formiate" buffer (80:20 v/v, pH 4). Two extraction fractions (F1a/D2-1, F1a/B3-1) were prepared by evaporating the solvent. The corresponding nonextractable residues were labeled F1a/D2-R and F1a/B3-R. Radioactivity in all of these fractions was determined. For metabolite identification,

the fractions were analyzed by TLC using reference standards (CGA 354743, CGA 368208, and CGA 357704).

Cage wash - Cage wash samples were mixed with scintillation fluid (Irgasafe Plus, Packard Instrument Co.) and counted.

Tissues/carcass - The carcasses were homogenized in a food processor with dry ice. Samples of the carcass homogenates were combusted and analyzed by LSC.

4. Analytical techniques

LSC - LSC was performed using a Packard Tri-Carb, model 2000CA that computed quench-corrected distintegrations per minute (dpm).

Radio-TLC - The pattern of radioactivity detection on thin layer plates was accomplished by use of a spark chamber radiochromatogram camera (Berta; Raytest, Straubenhardt, Germany). Quantitation of the fractions was performed by extraction of each zone on the thin layer plate and subsequent radioassay by LSC. For isolation of metabolites, the thin layer plates were analyzed by a Bio-Imaging Analyzer (BAS 2000; Fuji Photo Film Co., Ltd. Tokyo, Japan) with quantitation of radioactivity by TINA (Raytest, Straubenhardt, Germany) software that provided percent of total radioactivity on the plate.

Radio-HPLC - The HPLC eluent fractions were collected and aliquots of each fraction assayed by LSC.

Thin Layer Chromatography (TLC) - TLC was performed using precoated silica gel 60 F_{254} and RP-18 F_{254s} plates. Specific solvent systems and stationary phases were used for determination of the stability of CGA 77102, analysis of metabolite patterns, and isolation of metabolites (these have not been duplicated in this DER but are shown in tabular form on pp. 21-12 of the study report (MRID 44931715). Non-radioactive fractions on thin layer plates were visualized by dark quenching spots against the fluorescent background under UV light (254 nm). Rf values for the various reference standards were also provided on p. 22 of the study report.

High Performance Liquid Chromatography (HPLC) - HPLC analysis used a System Gold Nouveau system (Beckmann Instruments, Inc., San Ramon, CA) equipped with UV and radioactivity detectors. For analysis of urine samples, the system used a C-18 Nucleosill 120/5 µM 250 x 4 mm column, 1 mL/min flow rate with detection at 230 nm. A gradient solvent system of aqueous ammonium formiate buffer (Solvent A) and acetonitrile (Solvent B) was used. Specifics for the gradient flow and the retention times for the reference standards were provided on p. 23 of the study report.

5. Histopathology

Histopathologic analysis was not a protocol component and not performed.

6. Statistics

Group means and standard deviations were determined. Calculation methods for determining percent of metabolites in feces and urine, and determination of detection limits in tissues were also provided.

II. RESULTS

A. DISTRIBUTION/EXCRETION STUDIES

1. Mass balance

Overall recovery of administered radioactivity was acceptable (95.05-99.18% for low dose and 93.83-95.66% for high dose). Mass balance data are summarized in Table 2.

TABLE 2. Overall recovery of administered radioactivity (% of dose) at 72 hours post dosing in rats given [Phenyl-U- ^{14}C] CGA 77102

	Single low dose		Single high dose	
	Male	**Female**	**Males**	**Females**
Expired air	Not measured	Not measured	Not measured	Not measured
Urine	30.22±0.36	42.13±4.70	32.03±5.58	41.98±6.32
Feces	56.50±7.00	49.58±5.28	54.50±8.87	47.35±5.93
Carcass	8.11±2.97	7.00±3.05	6.82±1.89	5.52±1.43
Cage wash	0.23±0.12	0.47±0.12	0.48±0.36	0.82±0.55
Total	95.05±9.06	99.18±2.72	93.83±2.50	95.66±1.00

Data taken from Tables 3- 6, pp. 34-35, MRID 44931715.

2. Absorption

Absorption of the test material may be implied from the urinary excretion data as shown to represent approximately 30-42% of the administered dose. Addition of carcass burden (assuming it did not represent unabsorbed radioactivity from the gastrointestinal lumen) contributed an additional 6-8%. Tissue distribution and biliary excretion data were not provided and, therefore, unavailable for assessing absorption. Absorption appeared to be independent of dose at the doses tested.

3. Excretion

Based upon radioactivity in the urine, feces and cage wash, 87-92% of the administered radioactivity was eliminated by 72 hours. For both low and high dose groups, urinary elimination accounted for approximately 30-42% and fecal elimination

accounted for 47-57% of the administered dose. Overall and route-specific elimination appeared to be independent of dose. Although somewhat greater overall excretion was observed for females than for males in both dose groups, the differences were not statistically significant and could be explained by variability in one test animal and the small sample size. Under the conditions of this study, elimination via the feces was slightly greater than elimination via the urine although this difference was not as evident for female rats. Both routes are considered major routes of elimination for orally administered CGA 77102 at the doses and treatment period examined. The study focused on metabolite identification; time-course data for elimination were not generated.

4. Tissue distribution

Carcass burden at 72 hours represented 7-8% (low dose) and 6-7% (high dose) of the administered radioactivity (Table 2). Distribution among specific tissues was not examined.

B. PHARMACOKINETIC STUDIES

Kinetic parameters were not a protocol component and, therefore, were not assessed.

C. METABOLITE CHARACTERIZATION STUDIES

1. Urine

Resin column fractionation yielded three fractions. One fraction (first methanol eluate) contained approximately 90% of the radioactivity and was used for metabolite isolation. CGA 357704, CGA 368208, and CGA 354743 were confirmed as urinary metabolites. Quantitation data for these metabolites are presented in Table 3.

TABLE 3. Quantitation of urinary metabolites (percent of administered dose) from rats given single a oral dose of CGA 77102.

Dose group	CGA 357704	CGA 354743	CGA 368208
Low dose	0.02	0.03	0.03
High dose	0.05	0.003	0.02

Data taken from p. 30, MRID 44931715.

2. Feces

Quantitative date for fecal metabolites are shown in Table 4. Somewhat greater amounts of CGA 357704 and CGA 354743 were detected in the feces than were found in the urine.

TABLE 4. Quantitation of fecal metabolites (percent of administered dose) from rats given single a oral dose of CGA 77102.			
Dose group	**CGA 357704**	**CGA 354743**	**CGA 368208**
Low dose	0.12	0.25	0.05
High dose	0.16	0.14	0.06

Data taken from p. 30, MRID 44931715.

Total excretion of CGA 357704 was 0.14% and 0.21% of the administered dose for the low- and high-dose groups, respectively. Total excretion of CGA 354743 was 0.28% (low dose) and 0.14% (high dose) and total excretion of CGA 368208 was 0.08% for both the low- and high-dose groups.

D. HISTOPATHOLOGY

Histopathologic evaluations were not performed.

III. DISCUSSION

A. DISCUSSION

In a metabolism study (MRID 44931715), groups of three male and three female rats were each given [phenyl-U-^{14}C] CGA 77102 (batch no. ILS-143.1; purity 98.9%) and non-radiolabeled CGA 77102 (batch no. AMS 757-101; purity 99.8%) to provide a total single oral dose of 0.5 or 100 mg/kg. Urinary and fecal excretion were monitored over 72 hours and major metabolites identified and quantified. The study focused on evaluating the presence of the metabolites CGA 354743, CGA 368208, and CGA 357704 in the excreta of rats.

There were no deaths or overt signs of toxicity attributed to the test material. Actual administered doses were 3-8% greater than nominal. Overall recovery of administered radioactivity was an acceptable 93.83-99.18%. There were no significant differences in recovery efficiency between the treatment groups or between genders. Urinary excretion and carcass burden data implied that absorption was approximately 38-49% of the administered dose. Urinary and fecal elimination represented most of the recovered radioactivity; fecal elimination somewhat more so. The available data suggested that urinary excretion was slightly greater in female rats than male rats (42% for low- and high-dose females compared to 30% and 32% for low- and high-dose males), and fecal elimination was expectedly less (approximately 13-15%) for females than for males. However, the small sample size (three rats per sex) precludes a definitive assessment of gender-specific differences in excretory pattern. Time-course data for absorption and excretion were not provided. Based upon the residual radioactivity in the carcasses, accumulation in the tissues was less than 10% of the administered dose at 72 hours after

administration. No time-course data were generated in this study so it is difficult to determine the potential for bioaccumulation.

Both urinary and fecal metabolites were identified and quantified. For both routes of elimination, three major metabolites were identified but none represented more than 0.25% of the administered dose. Characterization of these metabolites and comparison to known reference standards revealed them to be CGA 357704, CGA 354743, and CGA 368208. In the feces, CGA 357704 and CGA 354743 represented a notably greater percent of the administered dose than in the urine. The amounts of CGA 368208 were similar in the feces and urine. Biliary excretion experiments were not performed and, therefore, no assessment can be made regarding biliary or gut microflora as the source of these biotransformation products in the feces.

This metabolism study in rats is **Acceptable/Non-guideline**. Although not satisfying the requirements for a Metabolism and Pharmacokinetics Study [OPPTS 870.7485 (85-1)], this study (MRID 44931715) was properly designed and conducted, and provided supplemental data regarding the quantitation and identification of very low levels of urinary and fecal metabolites in rats given a single oral dose of CGA 77102.

B. STUDY DEFICIENCIES

The study utilized only three animals/sex/dose. Although this did not compromise the validity of the results or the overall conclusions of this supplementary study, it did prevent definitive statistical evaluation. The study was designed to specifically address quantitation and identification of urinary and fecal metabolites and not for Tier 1 data requirements, thus its Non-guideline classification.

DATA EVALUATION REPORT

PHENYL-U-[^{14}C]-CGA-376944 (METOLACHLOR DEGRADATE)

Study Type: METABOLISM AND PHARMACOKINETICS – RAT
[OPPTS 870.7485 (§85-1)]
MRIDs 44931716 and 44931717

Prepared for

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

Prepared by

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Disclaimer

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

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EPA Work Assignment Manager: Joycelyn Stewart, PhD Joycelyn Stewart, Date 6/8/2001
Toxicology Branch, Health Effects Division (7509C)

DATA EVALUATION RECORD

STUDY TYPE: Metabolism - Rat; OPPTS [870.7485 (§85-1)]

DP BARCODE: D260393 SUBMISSION CODE: S570059
P.C. CODE: 108801 (parent only) TOX. CHEM. NO.: 188DD

TEST MATERIAL (PURITY): Degradate of Metolachlor ([Phenyl-U-^{14}C]-CGA 376944) (chemical purity not specified); radiochemical purity >95.5%); unlabeled CGA-376944 (chemical purity not specified)

SYNONYMS: A sulfonic acid soil degradate of Metolachlor (CGA77102); S-[(2-Ethyl-6-methyl-phenyl)-2-methoxy-1-methyl-ethyl)-carbamoyl]-methanesulfonic acid-[Phenyl-U-^{14}C].

CITATION: Muller, T. (1997). Disposition of [Phenyl-U-^{14}C]-CGA-376944, a sulfonic acid soil metabolite of CGA-77102, in the rat. Novartis Crop Protection AG, CH-4002 Basle, Switzerland. Laboratory Study No. 030AM06, Novartis No. 795-97, November 25, 1997. MRID 44931717. Unpublished.

Hassler, S. (1999). Disposition of [Phenyl-U-^{14}C]-CGA-376944, a sulfonic acid soil metabolite of CGA-77102, in bile-duct cannulated rats after oral administration. Novartis Crop Protection AG, CH-4002 Basle, Switzerland. Laboratory Study No. 030AM08, Novartis No. 1066-99. MRID 44931716. Unpublished.

SPONSOR: Novartis Crop Protection, Inc., 410 Swing Road, Post Office Box 18300, Greensboro, NC 27419.

EXECUTIVE SUMMARY: In a metabolism study (MRIDs 44931716 and 44931717), groups of four male and female and six male Tif: RAI f (SPF) rats were given single oral doses of [Phenyl-U-^{14}C]-CGA-376944 (0.5 mg/kg nominal; Batch No. ILS-125.4 radiochemical purity >95.5%), for the metabolism and bile-duct cannulation studies, respectively.

There were no deaths or overt signs of toxicity that could be attributed to the test material. Weight loss in bile-duct cannulated rats was attributed to surgical trauma. Radioactivity inventory indicated an acceptable 96.46-99.01% recovery of the administered dose among the experimental groups.

Based on urinary excretion, biliary excretion, and carcass burden, 17.35% of the administered radioactivity was absorbed following a single oral dose of 0.5 mg/kg of [Phenyl-U-^{14}C]-CGA-376944. Absorption was rapid but limited and most of the absorbed radioactivity (92.3%) was excreted within 24 hours; primarily in the bile. At 72 hours, measurable radioactivity was found only in the liver of non-cannulated rats. Carcass burdens accounted for <0.01% of the administered dose at necropsy.

Fecal elimination was the major route of excretion, accounting for most of the administered oral dose in non-cannulated rats (94.24-96.27%). Fecal excretion was rapid with 98.8-99.2% of the fecal excretion occurring within 24 hours post-dosing. Urinary excretion, accounted for only 2.1-4.4% of the dose in non-cannulated rats and 5.3% in bile-duct cannulated rats. Urinary excretion was rapid and nearly complete within 24 hours of dosing. Biliary excretion represented 11.5% of the administered dose at 48 hours. The majority of biliary excretion (99.2%) occurred within 24 hours after dosing. In bile-duct cannulated animals, an additional 76.8% of the administered dose was excreted in the feces. Based on these data, biliary excretion is a contributor to fecal elimination of the test material. This appears consistent with the occurrence of enterohepatic circulation via the hepatic portal system and bile-duct. Only a minor percentage of the dose (5.3%) appeared to enter the systemic circulation where it was rapidly excreted by the kidneys. No biologically relevant gender-related differences were detected in the oral dose groups.

Blood pharmacokinetic parameters could not be calculated due to low blood concentrations and rapid clearance of the administered dose. Blood levels of radioactivity peaked in both sexes within one hour post-dosing.

It is evident from the results of this study that the test material undergoes limited but rapid absorption and nearly complete excretion within 24 hours. The primary route of excretion is via the feces with biliary excretion products representing about 15.0% of the fecal excretion products. At the dose tested, [^{14}C]-CGA-376944 exhibits little potential for accumulation in the tissues. There were no significant gender-related differences in absorption and disposition of the test material. The unchanged test material accounted for >90% of the dose in both males and females. The unknown metabolites of urine and feces, separated by TLC accounted for 0.2-2.8% of the administered radioactivity and were not characterized further.

This combined metabolism study in rats is **Acceptable/Guideline** and satisfies the requirements for a Metabolism and Pharmacokinetics study [OPPTS 870.7485 (§85-1)].

COMPLIANCE: Signed and dated Good Laboratory Practice, Quality Assurance, and Data Confidentiality statements were included. A flagging statement was not included but is not necessary.

I. MATERIALS AND METHODS

A. MATERIALS

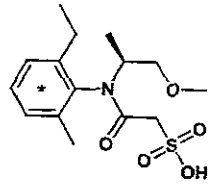
1. Test compound

Radiolabeled: [Phenyl-U-^{14}C]-CGA-376944 (Metolachlor degradate)
Batch No.: ILS 125.4
Radiochemical purity: >95.5%
Chemical purity: not specified
Description: not available
Contaminants: none noted
CAS No.: not available

Radiolabeled reference compound: [Phenyl-U-^{14}C]-CGA-354743 (radiolabeled racemic mixture of CGA-376944)
Batch No.: KI-5408/6
Radiochemical purity: 98%
Chemical purity: not specified
Description: not available
Contaminants: none noted
CAS No.: not available

Non-radiolabeled: CGA-376944 (Metolachlor degradate)
Purity: not specified
Batch No.: not available
Description: not given
Contaminants: none noted
CAS No.: not available
Stability: not stated

Non-radiolabeled CGA-354743 (racemic mixture of CGA-376944)
Purity: 99%
Batch No.: RV-2816/3
Description: not given
Contaminants: none noted
CAS No.: not available
Stability: not stated
Structure:



* = [^{14}C]

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2. Vehicle

Physiological saline (0.9% NaCl) for oral administration.

3. Test animals

Species: rat
Strain: Tif: RAI f (SPF)
Age and mean weight at study initiation: Metabolism: males 186 g at 7 weeks; females 182 g at 9 weeks. Bile-duct cannulation: males 256 g at 7 weeks.
Source: Biological Research Laboratory (BRL), Füllinsdorf Switzerland for metabolism study and RCC, Biotechnology and Breeding Division, Füllinsdorf, Switzerland for bile-duct cannulation study.
Housing: Polycarbonate cages in groups during acclimatization; individually in metabolism cages (open plexiglass for metabolism and non-restriction metabolism with a tail cuff and dual channel infusion catheter for bile-duct cannulation) during experiment.
Diet: powdered certified standard diet (Nafag No. 890, Nafag, Gossau, Switzerland) *ad libitum* .
Water: tap water *ad libitum*
Environmental conditions:
Temperature: metabolism 20°C; cannulated 22±2°C
Humidity: metabolism 42-68%; cannulated 53-80%
Air changes: not specified
Photoperiod: 12 hour light/dark cycle
Acclimation period: ≥4 days prior to metabolism study; ≥5 days prior to bile-duct cannulation surgery.

4. Preparation of dosing solution

The test doses were prepared by dissolving the labeled test material in water and diluting with 0.9% saline to a concentration of 0.133 mg/mL. Animals were administered approximately 0.8 mL each, resulting in males receiving 0.57 mg/kg, females 0.58 mg/kg, and bile-duct cannulated males 0.49 mg/kg. Stability was determined at the time of dosing. The administered dose was determined by diluting 3 gavage doses (0.8 mL) to 2 mL with water and determination of the radioactivity in 3 aliquots of each by liquid scintillation counting (LSC).

Results –
Homogeneity: Radioactivity analyses of dosing solution samples confirmed homogeneity (results were not provided).
Stability: Test article was stable at the time of administration and doses contained 97% of the test material as radiolabeled CGA- 376944; verified by TLC. This was considered adequate for the gavage dosing protocol.
Dose confirmation: Confirmed by radio analysis (results were not provided).

B. STUDY DESIGN AND METHODS

1. Group arrangements

Animals were assigned to experimental groups by random selection based upon age corresponding to approximately 200 g for metabolism and 250 g for the bile-duct cannulation study. Conventional randomization procedures were not used. For the metabolism study, groups of 4 males and 4 females received nominal oral doses of 0.5 mg/kg and for the bile-duct cannulation study, 6 males received 0.5 mg/kg oral doses; all by gavage. The study protocols are summarized in Table 1.

TABLE 1. Study design

Experimental group	Dose (mg/kg)	Number/Sex	Remarks
Single low-dose (metabolism) (MRID 44931717)	0.5 mg/kg	4 males 4 females	Assessment of absorption, distribution, and excretion of test material; metabolite characterization
Single low-dose (biliary excretion) (MRID 44931716)	0.5 mg/kg	6 males	Assessment of biliary excretion of test material

Information taken from p. 15 (MRID 4493171516) and p. 14 (MRID 44931717).

2. Dosing and sample collection

Animals were given single oral doses for the metabolism and bile-duct cannulation studies. Bile-duct cannulated rats were dosed after recovery (≥18 hours) from the implantation surgery. To compensate for the bile collected from the bile-duct, an artificial bile fluid (0.05% bile salts dissolved in physiological saline) was infused into the duodenum at a flow rate of 0.8 mL/hr. The catheters were run subcutaneously and terminated in a cuff at the tail. From there the catheters were led by a steel spring to a dual channel swivel which allowed free movement within the plexiglass metabolism cage.

Expired air - Expired air was not collected.

Blood - Blood was collected from 3 animals of each sex by tail tip amputation at post-dosing intervals of 0.25, 0.5, 0.75, 1, 1.5, 2, 2.5, 3, 4, 8, 24, and 48 hours. Blood was not collected from bile-duct cannulated animals.

Bile - Bile was collected only from animals cannulated for that purpose. Samples were taken at intervals of 0-1, 1-2, 2-4, 4-8, 8-24, 24-32, and 32-48 hours post-dosing.

Urine - Urine was collected at 0-4, 4-8, 8-24, 24-48, and 48-72 hours for the male and female rats from the metabolism study and at 0-24 and 24-48 hour intervals for bile-duct cannulated male rats.

Feces - Feces were collected at 24-hour intervals (up to 48 hours post-dosing) for the bile-duct cannulated rats and at 0-8, 8-24, 24-48, and 48-72 hours for the conventional metabolism study.

Cage wash - Cages were washed with water/ethanol (1:1, v/v) at the end of the collection period for mass balance analysis.

Tissues - Animals were sacrificed by exsanguination following CO_2 anaesthesia. For the metabolism study, tissues were collected from males and females at the end of the scheduled sacrifice period (72 hours). The following tissues were collected: heart, liver, spleen, kidneys, bone, lungs, gonads, skeletal muscle, brain, adipose tissue, uterus, gastro-intestinal (GI) tract, and residual carcass. For bile-cannulated male rats, only the GI tract and the carcass were retained for analysis at sacrifice (48 hours).

3. Sample preparation/analysis

Collected urine, feces, bile, tissues, and organs were kept frozen, the collected blood was kept refrigerated and the cage washes were retained at ambient temperature until analysis. Aliquots of liquid samples (blood, urine, bile, and cage washes) underwent no additional treatment and were mixed directly with scintillation cocktail (Irgasafe plus, Packard Instrument Co.). Feces was mixed with water and homogenized manually with a pestle. Bone was cut into pieces with a scissors as were brain, lungs and ovaries. Other tissues (2-4 subsamples) were combined into one aliquot. Carcasses and GI tracts were homogenized frozen in a food chopper. Samples of blood, bone, macerated lungs, GI tract, homogenized feces, carcass, and feces residues after extraction were placed in combustion cones and analyzed by combustion analysis. The resultant CO_2 was trapped in Carbosorb (Packard Instrument Co.) and mixed with Permaflour E+ (Packard Instrument Co.) for LSC. Aliquots of brain, heart, kidneys, liver, muscle ovaries, spleen, testes, and uterus were solubilized in Soluene (Packard Instrument Co.), neutralized with HCl and scintillation cocktail (Irgasafe plus) was added to samples for LSC.

4. Analytical techniques

Pooled urine samples from males and females and bile samples from bile-duct cannulated males were analyzed directly by thin layer chromatography (TLC) on silica gel 60 F_{254} plates (E. Merck AG.) developed with acetonitrile/water/formic acid 90:5:5 (v/v/v). The same solvent was used to determine the stability of CGA 376944 in the dosing solution using silica gel 60 F_{254} and RP-18 F_{254S} (E. Merck AG.). Pooled feces from male and female rats was mixed with acetonitrile at about 1:5 (w/v) and extracted twice by shaking followed by centrifugation. The pooled supernatants were reduced in volume and metabolites were characterized by TLC.

LSC samples were counted in a Packard Tri-Carb, model 2000A, liquid scintillation spectrometer with automatic quench correction. The radioactivity on (TLC) plates was detected using a model Berta spark chamber radiochromatogram camera

(Raytest) or a Packard Instant Imager. For quantification, radioactive zones were scraped off TLC plates, extracted with methanol, and counted in Irgasafe plus liquid scintillation cocktail. Non-radioactive fractions were visualized under UV irradiation (254 nm).

5. Histopathology

Histopathologic evaluations were not done as part of the metabolism and bile-duct cannulation studies.

6. Statistics

Statistical methods were not described. Data were presented as means plus or minus standard deviations, but no level of statistical significance was indicated.

II. RESULTS

A. DISTRIBUTION/EXCRETION STUDIES

1. Mass balance

Overall recovery of administered radioactivity was acceptable, ranging from 96.46 to 99.01% of the administered dose for the metabolism and bile-duct cannulation studies. Recovery of administered radioactivity was not significantly different among the treatment groups. Mass balance data are summarized in Table 2.

TABLE 2. Overall recovery of administered radioactivity (% of dose) in rats given a single oral dose of [Phenyl-U-^{14}C]-CGA-376944 at 0.5 mg/kg

	Metabolism study[a]		Bile-duct cannulation study[b]
	Male	Female	Male
Bile	n.a.	n.a.	11.51
Urine	2.05	4.39	5.34
Feces	96.27	94.24	76.78
Tissues	0.01	<0.01	n.a.
Carcass	<0.01	<0.01	0.49
GI tract	<0.01	<0.01	1.43
Cage wash	0.10	0.37	0.91
Total	98.43	99.00	96.46

n.a. = not applicable.
[a]0-72 hours.
[b]0-48 hours.
Data taken from Tables 3 and 4, pp. 28 and 29, MRID 44931717 and Table 3, page 31, MRID 44931716.

2. Absorption

Absorption of the test material from the gastrointestinal tract into enterohepatic circulation may be implied from the urinary excretion, biliary excretion, and residual carcass data following oral administration of the test material to bile-duct cannulated, male rats (Table 1). Rapid absorption following a single oral dose of 0.5 mg/kg resulted in recovery of 11.51, 5.34, and 0.49% of the administered radioactivity in the bile, urine, and carcass, respectively, at 48 hours post-dosing (17.34% total absorption). Within 24 hours of administration 92.4% of the absorbed dose was excreted in the urine (26.5%) and bile (65.9%).

3. Excretion

Time-course data for excretion via the urine, bile, and feces in the dose groups are given in Table 3.

Fecal excretion accounted for the majority of the recovered radioactivity and represented approximately 76.8% and 94.24-96.27% of the administered dose for the bile-duct cannulated animals and non-cannulated treatment groups, respectively. The observed difference in fecal excretion between cannulated and non-cannulated animals was due to separate collection of bile and feces in the cannulated animals. The total excretion in the cannulated rats is not significantly different from the non-cannulated rats and indicates that biliary excretion is responsible for a moderate amount (11.5%) of the total administered radiolabeled dose which is excreted via the feces in non-cannulated rats. The data also indicate that a significant portion of the dose (≈77%) recovered from feces is excreted directly without absorption. Gender-related differences were not biologically or statistically significant (Table 3). Biliary excretion and fecal excretion followed the same pattern in both studies with the majority of the dose being excreted within 24 hours after administration. The results appear consistent with the occurrence of enterohepatic recirculation.

Urinary excretion accounted for only minor amounts of the total administered radioactivity; 2.05-4.39% (non-cannulated) of the administered dose in 72 hours or 5.34% (cannulated) in 48 hours. The majority of the urinary excretion of the administered oral dose occurred within 24 hours (97% non-cannulated; 86% cannulated). Gender-related differences and differences between cannulated and non-cannulated urinary excretion in rats were not of biological significance.

4. Tissue distribution

Tissue burdens at 72 hours were minimal. The only tissue with radioactivity above the limit of determination was the liver from males (0.01% of total administered radioactivity). Carcass burden for non-cannulated rats was <0.01% and for cannulated rats was 0.49% of the administered oral dose. Because of the low levels found in tissue/carcass, a time-course analysis was not necessary.

TABLE 3. Time-course for excretion of administered radioactivity (% of dose) in rats given a single oral dose of [Phenyl-U-^{14}C]-CGA-376944 at 0.5 mg/kg

Sample/Time (hrs)		Metabolism study[a]		Bile-duct cannulation study[b]
		Male	Female	Male
Bile	0-1	n.a.	n.a.	0.41
	1-2	n.a.	n.a.	1.40
	2-4	n.a.	n.a.	3.19
	4-8	n.a.	n.a.	3.29
	8-24	n.a.	n.a.	3.13
	24-32	n.a.	n.a.	0.03
	32-48	n.a.	n.a.	0.06
Subtotal		n.a.	n.a.	11.51
Urine	0-4	0.88	1.37	n.a.
	4-8	0.71	0.67	n.a.
	8-24	0.40	2.20	n.a.
	0-24	n.a.	n.a.	4.59
	24-48	0.04	0.12	0.75
	48-72	0.02	0.04	n.a.
Subtotal		2.05	4.39	5.34
Feces	0-8	31.34	19.48	n.a.
	8-24	64.13	73.53	n.a.
	0-24	n.a.	n.a.	59.51
	24-48	0.76	1.16	17.28
	48-72	0.03	0.07	n.a.
Subtotal		96.27	94.24	76.78
Cage wash[c]		0.10	0.37	0.91
Total excreted		98.41	99.00	94.54

n.a. = not applicable.
[a]0-72 hours.
[b]0-48 hours.
[c]Performed only at termination.
Data taken from Tables 3 and 4, pp 28 and 29, MRID 44931717 and Table 3, page 31, MRID 44931716.

B. PHARMACOKINETIC STUDIES

Blood levels of radioactivity peaked in both males and females within one hour post-dosing. However, plasma concentrations barely exceeded the limit of determination (≈0.005 ppm CGA-376944 equivalents) in male and female rats. Maximal concentration (0.019 ppm) was reached in only one male and female at 15 minutes post-dosing. Pharmacokinetic parameters could not be calculated due to the low levels of radiolabel detected and the short retention times encountered. Rapid excretion of absorbed material in the bile is assumed to account for the low blood residue levels observed.

C. METABOLITE CHARACTERIZATION STUDIES

Pooled urine (0-48 hours) was analyzed by one-dimensional TLC. A pattern of 5 metabolite fractions was observed. One fraction, which co-chromatographed with CGA-376944, accounted for 17.9% of the urine radioactivity, but only 0.96% of the administered dose. The remaining four unknown fractions accounted for 20.8, 52.8, 3.8, and 7.5% of the radioactivity in urine and, respectively, 1.1, 2.8, 0.2, and 0.4% of the radioactivity in the administered dose.

Pooled bile fluid (0-48 hours) was analyzed by one-dimensional TLC. The major fraction, which co-chromatographed with CGA-376944, accounted for 80% of the radioactivity in the bile (9.2% of the administered dose). The remaining 3 unknown fractions accounted for 4.3, 13.0, and 2.6% of the biliary radioactivity and, respectively, 0.5, 1.5, and 0.3% of the radioactivity in the administered dose.

The majority of the administered radioactivity was recovered from total feces; 96.27 and 94.24% in male and female non-cannulated rats, respectively. Bile and feces accounted for 11.51 and 76.78%, respectively, of the administered radioactivity recovered from bile-duct cannulated males (88.29% total). Samples (0-24 hour pooled) were analyzed by one-dimensional chromatography following extraction with acetonitrile. Extraction yielded 96.6 and 97.3% of the total radioactivity in the pooled feces for male and female rats, respectively (non-cannulated). The sole metabolite recovered co-chromatographed with CGA-354743 (racemic mixture of CGA-376944). The unchanged test substance accounted for >90% of the dose in both males and females. The unknown metabolites of urine and feces, separated by TLC accounted for 0.2-2.8% of the administered radioactivity and were not characterized further.

D. HISTOPATHOLOGY

Histopathologic assessment was not a component of the reviewed studies (MRIDs 44931716 and 44931717).

III. DISCUSSION

A. DISCUSSION

There were no deaths or overt signs of toxicity that could be attributed to the test material. Bile-duct cannulated males lost 9-20 g between dosing and necropsy. This was attributed to surgical trauma and after treatment; not to test article toxicity. Radioactivity inventory indicated an acceptable recovery (96.46-99.01%) of the administered dose among the experimental groups.

Based on urinary excretion, biliary excretion, and carcass burden, 17.34% of the administered dose of [Phenyl-U-^{14}C]-CGA-376944 was absorbed following a single oral dose of 0.5 mg/kg. Essentially all of the absorption occurred during the first 24 hours after administration. Deposition of test material-related radioactivity into tissues was minimal.

At 72 hours post dosing, overall tissue/carcass burdens represented <0.01 and 0.01% of the administered oral doses for non-cannulated males and females, respectively. For bile-duct cannulated males, 1.92% if the administered radioactivity was recovered in the GI tract and carcass at 48 hours post-dosing. Most tissues examined did not contain detectable levels of the test material.

Fecal elimination was the major route of excretion, accounting for most of the administered oral dose in non-cannulated rats (94.24-96.27%). Fecal excretion was rapid with 98.8-99.2% of the fecal excretion occurring within 24 hours after dosing. Contributions to fecal excretion were negligible after 48 hours. No biologically relevant, gender-related differences were detected in the oral dose groups. Urinary excretion accounted for approximately 2.1-4.4% of the oral dose. Similar to fecal excretion, urinary excretion was rapid and nearly complete within 24 hours of dosing. Only minor amounts of radioactivity were excreted in the urine after 48 hours. Biliary excretion represented 11.51% of the administered dose at 48 hours. The majority of the biliary excretion (99.2% of total recovered in bile) occurred within 24 hours after dosing. In the cannulated animals, 76.78% of the administered dose was excreted in the feces and 5.34% in the urine. Based on these data, biliary excretion is a contributor to fecal elimination of the test material. This appears consistent with the occurrence of enterohepatic recirculation via the hepatic portal system and bile duct. Only a minor percentage of the dose (5.34%) appeared to enter the systemic circulation where it was rapidly excreted via the kidneys.

Tissue/carcass burdens at 72 hours, after a single oral dose, represented only <0.01-0.01% of the total administered radioactivity. Tissues accounted for ≈0.01% and carcasses accounted for <0.01% of the administered dose. Measurable levels of radioactivity were found only in the liver of non-cannulated male rats.

Blood levels of radioactivity peaked in both males and females within one hour post-dosing. Radioactivity barely exceeded the limit of determination in blood (≈0.005 ppm CGA-376944 equivalents); maximal concentration (0.019 ppm) was reached in only one male and female at 15 minutes post-dosing. No blood pharmacokinetic parameters were calculated due to low blood levels and short residence times for the test material. Dispositional processes were not tested for saturation (multiple doses or a dose range not tested).

It is evident from the results of this study that the test material undergoes limited but rapid absorption and nearly complete excretion within 24 hours. The primary route of excretion is via the feces with biliary excretion products representing about 15.0% of the fecal excretion products. At the dose tested, [^{14}C]-CGA-376944 exhibits little potential for accumulation in the tissues. There were no significant gender-related differences in absorption and disposition of the test material. The unchanged test material accounted for >90% of the dose in both males and females. The unknown metabolites of urine and feces, separated by TLC, accounted for 0.2-2.8% of the administered radioactivity and were not characterized further.

Although some deficiencies were noted, this metabolism study in rats is **Acceptable/Guideline** and satisfies the requirements for a Metabolism and Pharmacokinetics study [OPPTS 870.7485(§85-1)].

B. STUDY DEFICIENCIES

The experimental protocols of the reviewed studies (MRIDs 44931716 and 44931717) were established to examine the absorption, distribution, excretion, plasma kinetics, and metabolite characterization of the test material following single oral doses in male and female rats as well as excretion and metabolite characterization in bile-duct cannulated male rats. The data obtained were adequate to meet these objectives. However, some deficiencies were noted which did not affect the conclusions drawn from the study data. No pretest toxicity study was included. The doses appeared to consist of the radiolabeled compound only without dilution by unlabeled test material, and the radiochemical purity was stated but the chemical purity was not. The dose administered (0.5 mg/kg) was quite low with no rationale provided. It is possible that a low dose was selected to represent the expected concentration of the test material in soil as a result of aerobic and/or anaerobic metabolism of the parent (Metolachlor). Because only one dose was tested, assessing the effect of dose and dosing protocol on absorption, excretion, or metabolism patterns was not possible. The data set, therefore, is considered minimal.

DATA EVALUATION REPORT

CGA-354743
(CGA-354743 TECHNICAL)

STUDY TYPE: ACUTE ORAL TOXICITY - RAT [870.1100 (81-1)]
MRID 44991101

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. 00-09.1A

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Disclaimer

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

Oak Ridge National Laboratory, managed by Lockheed Martin Energy Research Corp. for the U.S. Department of Energy under contract number DE-AC05-96OR22464.

EPA Reviewer: Virginia A. Dobozy, VMD, MPH Virginia A Dobozy, Date 4/11/01
Reregistration Branch I, Health Effects Division (7509C)
EPA Work Assignment Manager: Joycelyn Stewart, PhD Joycelyn Stewart, Date 4/26/01
Toxicology Branch, Health Effects Division (7509C)

DATA EVALUATION RECORD

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

DP BARCODE: D262025 SUBMISSION CODE: S572956
P.C. CODE: 108801 (Metolachlor) TOX. CHEM. NO.: 188DD

TEST MATERIAL (PURITY): CGA-354743 Tech. (Metabolite of CGA-24705, 95% a.i.)

SYNONYMS: not reported

CITATION: Winkler, G. (1995) Acute oral toxicity in the rat (limit test). Short-term Toxicology, Novartis Crop Protection, Inc. (Formerly Ciba-Geigy Limited), 4332 Stein, Switzerland. Laboratory study identification 816-95, December 5, 1995. MRID 44991101. Unpublished.

SPONSOR: Novartis Crop Protection, Inc., 420 Swing Road, P.O. Box 18300, Greensboro, NC 27419

EXECUTIVE SUMMARY: In an acute oral toxicity study (MRID 44991101) five male and five female fasted young adult Tif:RAI f (SPF) rats were given a single oral 2000 mg/kg dose of CGA-354743 Tech. (95%, a.i., Batch No. RV-2816/1) in 0.5% w/v Carboxymethylcellulose in 0.1% w/v aqueous polysorbate 80 and observed for 14 days.

No animals died during the study. Piloerection and hunched posture were noted from all rats one hour post dosing with recovery by day 3. With the exception of one female that lost weight during the second week, all rats had normal body weight gains. No observable abnormalities were noted at necropsy.

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

CGA-354743 Tech. is in TOXICITY CATEGORY III based on the LD_{50}.

This acute oral study is classified as **Acceptable/Guideline** and satisfies the 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: CGA-354743 Tech. (Metabolite of CGA 24705)

   Description: solid
   Lot/Batch #: RV-2816/1
   Purity: 95% a.i.
   CAS #: 51218-45-2 (Metolachlor)

2. Vehicle and/or positive control

   0.5% w/v Carboxymethylcellulose in 0.1% w/v aqueous polysorbate 80

3. Test animals

   Species: rat
   Strain: Tif:RAI f (SPF)
   Age and/or weight at dosing: young adult; males: 219-229 g, females: 191-205 g
   Source: Ciba-Geigy Limited, Laboratory Animal Breeding, Pharma Division, 4332 Stein, Switzerland
   Acclimation period: at least 5 days
   Diet: NAFAG 890 (NAFAG, Gossau/SG, Switzerland), *ad libitum*
   Water: *ad libitum*
   Housing: five animals per Macrolon cages type 4
   Environmental conditions:
   Temperature: 22±2°C
   Humidity: 55±10%
   Air changes: approximately 15/hour
   Photoperiod: 12 hour light/dark

B. STUDY DESIGN AND METHODS

1. In life dates

   Start: October 19 (males) and 24 (females), 1995; end: November 7, 1995

2. Animal assignment and treatment

   Following an overnight fast, five rats/sex were given a single 2000 mg/kg dose of the test material in 0.5% w/v Carboxymethylcellulose in 0.1% w/v aqueous polysorbate 80 by gavage. The animals were observed for clinical signs of toxicity daily and for mortality twice daily for 14 days. They were weighed immediately 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 CGA-354743 tech. toxicity.

The oral LD_{50} for males, females, and combined was > 2000 mg/kg. This places CGA-354743 Tech. in TOXICITY CATEGORY III.

B. CLINICAL OBSERVATIONS

Piloerection and hunched posture were noted from all rats one hour post dosing with recovery by day 3.

C. BODY WEIGHT

With the exception of one female that lost weight during the second week, all rats had normal body weight gains.

D. NECROPSY

No observable abnormalities were noted.

E. DEFICIENCIES

None

DATA EVALUATION REPORT

CGA-354743

STUDY TYPE: MAMMALIAN CELLS IN CULTURE GENE MUTATION ASSAY IN CHINESE HAMSTER V79 CELLS (OPPTS 870.5300) [§84-2]
MRID 44991102
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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Disclaimer

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

Oak Ridge National Laboratory, managed by Lockheed Martin Energy Research Corp. for the U.S. Department of Energy under contract number DE-AC05-96OR22464.

EPA Reviewer: Virginia A. Dobozy, VMD, MPH Virginia A Dobozy, Date 6/4/01
Reregistration Branch I, Health Effects Division (7509C)
EPA Work Assignment Manager: Joycelyn Stewart, PhD Joycelyn Stewart, Date 6/5/2001
Toxicology Branch, Health Effects Division (7509C)

DATA EVALUATION RECORD

STUDY TYPE: Mammalian cells in culture gene mutation assay in Chinese hamster V79 cells; OPPTS 870.5300 [§84-2]

DP BARCODE: D262025
P.C. CODE: 108801 (parent)

SUBMISSION CODE: S572956
TOX. CHEM. NO.: 188DD

TEST MATERIAL (PURITY): CGA-354743 tech. (cga-354743 (Metolachlor ESA, degretate of metolachlor), 98% a.i.)

SYNONYMS: none provided

CITATION: Ogorek, B. (1999) CGA-354743 tech. (Metabolite of CGA-24705): Final report - Gene mutation test with Chinese hamster cells V79. Genetic Toxicology, Novartis Crop Protection AG, CH-4002 Basel, Switzerland. Laboratory Study ID 981018, Novartis No. 1193-98, January 19, 1999. MRID 44991102. Unpublished.

SPONSOR: Novartis Crop Protection, Inc., 410 Swing Road, Post Office Box 18300, Greensboro, NC 27419

EXECUTIVE SUMMARY: In a mammalian cell gene mutation assay at the HPRT locus (MRID 44991102), Chinese hamster V79 cells in culture were exposed to CGA-354743 tech. in bidistilled water at concentrations of 185.19, 555.56, 1666.67, 5000.00 µg/mL in the presence and absence of mammalian metabolic activation (S9-mix). The S9-fraction was obtained from Aroclor 1254 induced male Tif:RAI/SPF rat liver.

CGA-354743 tech. was tested up to a weakly cytotoxic limit concentration of 5000 µg/mL. In a preliminary cytotoxicity test, the number of viable cells was reduced approximately 27% and 32%, compared to the solvent control group, at 5000.00 µg/mL with and without S9-mix, respectively. An initial and a confirmatory assay were conducted using two cultures per dose, four dishes per culture. In the presence of S9-mix, small but statistically significant increases in mean mutant frequencies over the solvent control value were seen in the initial mutation assay at concentrations of 555.56 µg/mL (4.10 per 10^6 viable cells, $0.002<p<0.01$) and 5000.00 µg/mL (5.35 per 10^6 viable cells, $p<0.001$) but not at 1666.67 µg/mL (3.17 per 10^6 viable cells). The mutant frequency of the solvent control was 2.80×10^6 viable cells. Results in the confirmatory assay with S9-mix were similar with small but statistically significant increases in mean mutant frequencies over the solvent control value of 1.58 per 10^6 viable cells seen at 555.56 µg/mL (2.60 per 10^6 viable cells, $0.02<p<0.05$), 1666.67 (3.40 per 10^6 viable cells, $0.002<p<0.01$) and 5000.00 µg/mL (2.91 per 10^6 viable cells, $0.002<p<0.01$). The mean mutant frequency of the

DMN positive control was 118.27 per 10^6 viable cells in the initial assay and 116.68 per 10^6 viable cells in the confirmatory assays. In the absence of S9-mix, statistically significant increases in mutant frequencies were seen at 555.56 and 5000.00 µg/mL CGA-35473 but not at 1666.67 µg/mL in both the initial and the first confirmatory assays. In the initial assay, the mean mutant frequency at 5000.00 µg/mL was 19.7 per 10^6 viable cells ($p<0.001$) compared to the solvent control value of 3.66 per 10^6 viable cells and the normalized mean number of mutants per flask was 39.41 compared to the solvent control value of 7.33. The results at 5000.00 µg/mL met the laboratory's criteria for a positive response. In the first confirmatory assay, the statistically significant increase in the mutant frequency over the solvent control value seen at 5000.00 µg/mL ($0.001<p<0.002$) was not accompanied by a normalized mean number of mutants per flask of at least 20 more than the solvent control (18.63 vs 6.63 for the solvent control), thus not meeting the criteria for a positive response. A second confirmatory assay was performed without S9-mix and small but statistically significant increases in mutant frequency were seen at 555.56 and 1666.67 µg/mL but not at 5000.00 µg/mL; therefore, the positive result seen in the initial assay could not be confirmed. There was a statistically significant relationship between dose and mutant frequency in the initial and first confirmatory assay ($p<0.001$) but not in the second confirmatory assay. Although the increases in mutant frequencies over solvent control values seen in this study were statistically significant, all mutant frequencies in test material treated cultures were well within the laboratory's historical solvent control range of 1.01 to 15.68 per 10^6 viable cells and below the historical solvent control mean mutant frequency of 5.46 per 10^6 viable cells (with the one exception at 5000.00 µg/mL without S9-mix). In addition, the solvent control values seen in both the initial and confirmatory assays were near the bottom of the historical control range. The normalized mean number of mutants per flask did not meet the laboratory criterion of 20 or more greater than the solvent control value at any dose with S9-mix or at any dose without S9-mix except at 5000.00 µg/mL as described. The statistically significant differences seen are thus unlikely to be biologically significant. It is of note that none of the assay results satisfied the generally accepted criteria for a positive response in this test system (i.e., reproducibility, dose response and/or minimum of 3-fold increase over background).[1] The positive and solvent controls induced the appropriate response. **There was suggestive (statistical) evidence of a possible induction of mutant colonies over background; however, the results are unlikely to be biologically significant because the absolute numbers of mutant colonies were low and within the testing laboratory's historical solvent control ranges.**

This study is classified as **Acceptable/Guideline**. It satisfies the requirement for FIFRA Test Guideline OPPTS 870.5300 [§84-2] OPPTS 870.5300 for in vitro mutagenicity (mammalian forward gene mutation) data.

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

---

[1]Nestman, ER, Brillinger RL, Gilman JPW, Rudd CJ, Swierenga SHH (1991). Recommended protocols based on a survey of current practice in genotoxicity testing laboratories: II Mutation in Chinese hamster ovary, V79 Chinese hamster lung and L5178Y mouse lymphoma cells. Mutat Res 246:255-284.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: CGA-354743 tech

   Description: white powder
   Lot/Batch #: KI-5408/6
   Purity: 98% a.i.
   Stability of compound: stable
   CAS #: not provided
   Structure: not provided
   Solvent used: bidistilled water
   Other comments: none

2. Control materials:

   Negative: none
   Solvent/final concentration: bidistilled water
   Positive: (concentrations/solvent):
   Nonactivated conditions: ethyl methanesulfonate / 0.3 µL/mL / not given
   Activated conditions: N-nitrosodimethylamine (DMN) / 1.0 µL/mL / not given

3. Activation: S9 derived from male Tif:RAI/SPF rats

x Aroclor 1254	x induced	x rat	x liver
_ phenobarbital	_ non-induced	_ mouse	_ lung
_ none _ hamster _ other			
_ other	_ other		

   If other, describe below

   S9 mix composition:

S9-fraction	250.0 µL/mL
glucose-6-phosphate	10.0 µmol/mL
NADP	8.0 µmol/mL
$CaCl_2$	20.0 µmol/mL
$MgCl_2$	20.0 µmol/mL
Na_2HPO_4	1.0 µmol/mL
FCS	30.0 µL/mL

4. Test cells: mammalian cells in culture

   _ mouse lymphoma L5178Y cells
   _ Chinese hamster ovary (CHO) cells
   x V79 cells (Chinese hamster lung fibroblasts)

Properly maintained? Y
Periodically checked for Mycoplasma contamination? Y
Periodically checked for karyotype stability? Y
Periodically "cleansed" against high spontaneous background? Y

Media:

5. Locus examined:

__ thymidine kinase (TK)
Selection agent: _____ bromodeoxyuridine (BrdU)
(give concentr.) _____ fluorodeoxyuridine (FdU)
_____ trifluorothymidine (TFT)

_x_ hypoxanthine-guanine-phosphoribosyl transferase (HPRT)
Selection agent: _____ 8-azaguanine (8-AG)
(give concentr. _8 µg/mL_ 6-thioguanine (6-TG)

__ Na^+/K^+ ATPase
Selection agent: _____ ouabain
(give concentration)

__ other (locus and/or selection agent; give details):

6. Test compound concentrations used

Preliminary cytotoxicity test:
Nonactivated and activated conditions: 2.44, 4.88, 9.77, 19.53, 39.06, 78.12, 156.25, 312.50, 625.00, 1250.00, 2500.00, 5000.00 µg/mL

Mutation assays (original and confirmatory):
Nonactivated and activated conditions: 185.19, 555.56, 1666.67, 5000.00 µg/mL

B. TEST PERFORMANCE

1. Cell treatment:

a. Cells exposed to test compound, negative/solvent or positive controls for: _21_ hours (nonactivated) _5_ hours (activated)

b. After washing, cells cultured for _7 - 8_ days (expression period) before cell selection:

c. After expression, 2 x 10^6 cells/dish (4 dishes/group/duplicate culture) were cultured for 7 - 8 days in selection medium to determine numbers of mutants and 100 cells/dish (6 dishes/group/duplicate culture) were cultured for 7 - 8 days without selective agent to determine cloning efficiency.

2. Statistical methods

Statistical significance of mutant frequencies (analysis of variance and test for linear trend) was carried out according to the UKEMS guidelines (Arlett et.al., 1990).

3. Evaluation criteria

Mutant frequencies were normalized to a virtual cloning efficiency of 100% at the end of the expression period. Mutant frequencies were usually not calculated for a culture if the cloning efficiency was lower than 15%. A mean mutant factor, defined as the ratio of the mean mutant frequencies of the treated cultures with the mean mutant frequencies of the solvent control cultures, was calculated for every concentration. Criteria for a positive response were (1) a mutant frequency at one or more concentrations significantly greater than that of the solvent control with the number of mutant clones in the treated and untreated cultures differing by more than 20, (2) a significant dose-relationship as indicated by the linear trend analysis, (3) reproducible results.

II. REPORTED RESULTS

The stability and intended concentrations of the test material were confirmed by analysis using HPLC with UV detection.

A. PRELIMINARY CYTOTOXICITY ASSAY

Twelve concentrations of CGA-354743 tech. ranging from 2.44 to 5000.00 µg/mL were tested, with and without S9-mix, in the preliminary cytotoxicity assay. Weak, concentration related growth inhibition was seen both with and without S9-mix. The number of viable cells was reduced approximately 27% and 32%, compared to the solvent control group, at 5000.00 µg/mL with and without S9-mix, respectively. The highest dose selected for testing in the mutation assay was 5000.00 µg/mL. Results of the cytotoxicity assay are presented in Appendix Tables 1 and 2 (MRID 44991102, pp. 28 and 29).

B. Mutagenicity assay

Four concentrations of CGA-354743 tech. ranging from 185.19 to 5000.00 µg/mL were tested, with and without S9-mix, in an initial and a confirmatory mutation assay. Two cultures per dose, four dishes per culture were evaluated. In the **presence of S9-mix**, small but statistically significant increases in mean mutant frequencies over the solvent control value were seen in the initial mutation assay at concentrations of 555.56 (4.10 per

10^6 viable cells, $0.002<p<0.01$) and 5000.00 µg/mL (5.35 per 10^6 viable cells, $p<0.001$) but not at 1666.67 µg/mL (3.17 per 10^6 viable cells). The mutant frequency of the solvent control was 2.80 x 10^6 viable cells. Results in the confirmatory assay with S9-mix were similar with small but statistically significant increases in mean mutant frequencies over the solvent control value of 1.58 per 10^6 viable cells seen at 555.56 µg/mL (2.60 per 10^6 viable cells, $0.02<p<0.05$), 1666.67 (3.40 per 10^6 viable cells, $0.002<p<0.01$) and 5000.00 µg/mL (2.91 per 10^6 viable cells, $0.002<p<0.01$). The mean mutant frequency of the DMN positive control was 118.27 per 10^6 viable cells in the initial assay and 116.68 per 10^6 viable cells in the confirmatory assays. There was a statistically significant positive linear relationship seen between dose and mutant frequency in the initial assay ($p<0.001$) and in the confirmatory assay ($0.01<p<0.025$). Although the increases in mutant frequencies over solvent control values were statistically significant, all mutant frequencies in test material treated cultures were well within the laboratory's historical solvent control range of 1.01 to 15.68 per 10^6 viable cells and below the historical solvent control mean mutant frequency of 5.46 per 10^6 viable cells. In addition, the solvent control values seen in both the initial and confirmatory assays were near the bottom of the historical control range. The normalized mean number of mutants per flask did not meet the laboratory criterion of 20 or more greater than the solvent control value at any dose. The statistically significant differences seen are thus unlikely to be biologically significant. Results of the initial and confirmatory assays with S9-mix are summarized in Appendix Tables 3 and 4, respectively (MRID 44991102, pp. 30 and 32).

Small but statistically significant increases in mutant frequencies were also seen in the **absence of S9-mix** in both the initial and in two confirmatory assays. In both the initial and the first confirmatory assays, statistically significant increases in mutant frequencies were seen at 555.56 and 5000.00 µg/mL but not at 1666.67 µg/mL. In the initial assay, the mean mutant frequency at 5000.00 µg/mL was 19.7 per 10^6 viable cells ($p<0.001$) compared to the solvent control value of 3.66 per 10^6 viable cells and the normalized mean number of mutants per flask was 39.41 compared to the solvent control value of 7.33. The results at 5000.00 µg/mL met the laboratory's criteria for a positive response. In the first confirmatory assay, the statistically significant increase in the mutant frequency over the solvent control value seen at 5000.00 µg/mL ($0.001<p<0.002$) was not accompanied by a normalized mean number of mutants per flask at least 20 more than the solvent control (18.63 vs 6.63 for the solvent control), thus not meeting the criteria for a positive response. A second confirmatory assay was performed without S9-mix and small but statistically significant increases in mutant frequency were seen at 555.56 and 1666.67 µg/mL but not at 5000.00 µg/mL; therefore, the positive result seen in the initial assay could not be confirmed. There was a statistically significant relationship between dose and mutant frequency in the initial and first confirmatory assay ($p<0.001$) but not in the second confirmatory assay. As discussed in the preceding paragraph describing the assays conducted with S9-mix, the statistically significant differences seen in the absence of S9-mix are unlikely to be biologically significant. Results of the assays without S9-mix are summarized in Appendix Tables 5 - 7 (MRID 44991102, pp.31, 33 and 34).

III. REVIEWER'S DISCUSSION/CONCLUSIONS:

A. This is an acceptable study. CGA-354743 tech. was tested up to a weakly cytotoxic limit concentration of 5000.00 µg/mL, suitable experimental protocol was followed and the positive and solvent control values were appropriate and within the laboratory's historical control ranges. What conclusions can be drawn from the study are, however, debatable. Five assays were conducted as part of this study, all using the same four concentrations of test material. Two assays were conducted with S9-mix and three without S9-mix. A small but statistically significant increase in mutant frequency over the solvent control value was seen at two or more doses in all five assays and a statistically significant positive relationship between dose and mutant frequency was seen in four of the assays. No statistically significant increases in mutant frequency were seen at the low dose in any of the five assays but were seen in four of the five assays at the high dose. These results seem to suggest that CGA-354743 tech. is weakly mutagenic in this assay system; however, with one exception (5000.00 µg/mL without S9-mix in the initial assay), the mean mutant frequencies were within the laboratory's historical solvent control range and the difference between the normalized mean number of mutants per flask in the solvent controls and test material treated cells was small (less than 20). The laboratory's criteria for a positive response were met at 5000.00 µg/mL without S9-mix in the initial assay but were not met in the first confirmatory assay although the mean mutant frequency at 9.31 per 10^6 viable cells and the normalized mean number of mutants per flask at 18.63 were both the second highest values seen in the study. There was no statistically significant increase in mutant frequency at this concentration in a second confirmatory assay. The study author concluded that there was no biologically significant increase in mutant frequency found in this study. The reviewers conclude that there is suggestive but not conclusive evidence of a very weak mutagenic effect in this study.

B. STUDY DEFICIENCIES

No Study Deficiencies Were Identified.

REFERENCES

Arlett, C.F., D.M. Smith, M.H. Green, D.B. McGregor, G.M. Clarke, J. Cole and J.C. Asquith (1990) Mammalian cell gene mutation assays based upon colony formation. In: Statistical Evaluation of Mutagenicity Test Data (ed. Kirkland, D.J.), Cambridge University press, pp. 66-101.

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APPENDIX

(MRID 44991102)

TABLE 1 : RESULT OF THE CYTOTOXICITY TEST
Experiment with metabolic activation

Test number : 981018
Experiment : Original
Test substance : CGA 354 743 tech.
Batch : KI-5408/6

Treatment	Cell number after treatment (x10E6)	Survival clones after treatment (per well)					
Negative control	1.420	84	86	81	79	87	84
Negative control	1.456	87	80	78	81	82	85
CGA 354 743 tech.:							
5000.0000 µg/ml	1.360	61	64	63	66	62	64
2500.0000 µg/ml	1.481	60	61	72	69	64	63
1250.0000 µg/ml	1.444	65	66	72	72	70	67
625.0000 µg/ml	1.343	60	64	66	71	70	69
312.5000 µg/ml	1.476	68	72	70	71	67	73
156.2500 µg/ml	1.493	67	64	69	73	66	64
78.1250 µg/ml	1.449	78	82	77	80	81	84
39.0625 µg/ml	1.371	76	81	81	74	73	72
19.5313 µg/ml	1.370	72	81	76	74	73	71
9.7656 µg/ml	1.356	79	76	77	81	80	84
4.8828 µg/ml	1.491	76	78	84	83	83	81
2.4414 µg/ml	1.282	81	86	77	79	84	84

Treatment	Mean of clones	Number of viable cells (x10E6)	Acute cytotoxicity (% of control)
Negative control	83.50	1.19	
Negative control	82.17	1.20	
CGA 354 743 tech.:			
5000.0000 µg/ml	63.33	0.86	27.70
2500.0000 µg/ml	64.83	0.96	19.40
1250.0000 µg/ml	68.67	0.99	16.73
625.0000 µg/ml	66.67	0.90	24.83
312.5000 µg/ml	70.17	1.04	13.01
156.2500 µg/ml	67.17	1.00	15.78
78.1250 µg/ml	80.33	1.16	2.23
39.0625 µg/ml	76.17	1.04	12.33
19.5313 µg/ml	74.50	1.02	14.28
9.7656 µg/ml	79.50	1.08	9.51
4.8828 µg/ml	80.83	1.21	nTx
2.4414 µg/ml	81.83	1.05	11.94

TABLE 2 : RESULT OF THE CYTOTOXICITY TEST
Experiment without metabolic activation

Test number : 981018
Experiment : Original
Test substance : CGA 354 743 tech.
Batch : KI-5408/6

Treatment	Cell number after treatment (x10E6)	Survival clones after treatment (per well)
Negative control	1.670	102 111 99 97 103 106
Negative control	1.444	104 110 101 98 106 102
CGA 354 743 tech.:		
5000.0000 µg/ml	1.480	70 71 81 74 76 73
2500.0000 µg/ml	1.688	72 76 80 77 78 74
1250.0000 µg/ml	1.694	76 75 81 82 78 77
625.0000 µg/ml	1.639	87 88 82 90 84 86
312.5000 µg/ml	1.525	96 91 90 88 88 88
156.2500 µg/ml	1.695	97 90 91 96 94 98
78.1250 µg/ml	1.643	91 86 87 89 92 92
39.0625 µg/ml	1.718	90 87 91 89 86 94
19.5313 µg/ml	1.585	96 96 90 89 97 96
9.7656 µg/ml	1.651	90 96 94 97 100 93
4.8828 µg/ml	1.389	92 96 99 93 90 95
2.4414 µg/ml	1.463	99 101 96 104 107 108

Treatment	Mean of clones	Number of viable cells (x10E6)	Acute cytotoxicity (% of control)
Negative control	103.00	1.72	
Negative control	103.50	1.49	
CGA 354 743 tech.:			
5000.0000 µg/ml	74.17	1.10	31.71
2500.0000 µg/ml	76.17	1.29	20.03
1250.0000 µg/ml	78.17	1.32	17.63
625.0000 µg/ml	86.17	1.41	12.16
312.5000 µg/ml	90.17	1.38	14.43
156.2500 µg/ml	94.33	1.60	0.54
78.1250 µg/ml	89.50	1.47	8.53
39.0625 µg/ml	89.50	1.54	4.36
19.5313 µg/ml	94.00	1.49	7.33
9.7656 µg/ml	95.00	1.57	2.43
4.8828 µg/ml	94.17	1.31	18.61
2.4414 µg/ml	102.50	1.50	6.69

TABLE 3 : SUMMARY OF THE MUTAGENICITY EXPERIMENT
Experiment with metabolic activation

Test number : 981018
Experiment : Original
Test substance : CGA 354 743 tech.
Batch : KI-5408/6

Treatment	Mean of viability clones per well	Mean of mutants per flask	Normalized mean of mutants per flask
Negative control	80.50	4.50	5.59
Positive control DMN 1 µl/ml	76.42	180.75	236.53
CGA 354 743 tech.:			
5000.0000 µg/ml	85.25	9.13	10.70
1666.6667 µg/ml	82.75	5.25	6.34
555.5556 µg/ml	82.25	6.75	8.21
185.1852 µg/ml	82.50	3.63	4.39

Treatment	Mean mutant frequency (x10E-6)	Mean mutant factor	Significance (P)
Negative control	2.80		
Positive control DMN 1 µl/ml	118.27	42.31	P<0.001
CGA 354 743 tech.:			
5000.0000 µg/ml	5.35	1.91	P<0.001
1666.6667 µg/ml	3.17	1.13	Ns
555.5556 µg/ml	4.10	1.47	0.002<P<0.01
185.1852 µg/ml	2.20	0.79	Ns

Linear relation: P<0.001

TABLE 5 : SUMMARY OF THE MUTAGENICITY EXPERIMENT
Experiment without metabolic activation

Test number : 981018
Experiment : Original
Test substance : CGA 354 743 tech.
Batch : KI-5408/6

Treatment	Mean of viability clones per well	Mean of mutants per flask	Normalized mean of mutants per flask
Negative control	73.33	5.38	7.33
Positive control EMS 0.3 µl/ml	65.58	682.75	1041.04
CGA 354 743 tech.:			
5000.0000 µg/ml	67.25	26.50	39.41
1666.6667 µg/ml	66.17	5.88	8.88
555.5556 µg/ml	71.58	8.75	12.22
185.1852 µg/ml	70.33	3.63	5.15

Treatment	Mean mutant frequency (x10E-6)	Mean mutant factor	Significance (P)
Negative control	3.66		
Positive control EMS 0.3 µl/ml	520.52	142.03	P<0.001
CGA 354 743 tech.:			
5000.0000 µg/ml	19.70	5.38	P<0.001
1666.6667 µg/ml	4.44	1.21	Ns
555.5556 µg/ml	6.11	1.67	0.01<P<0.02
185.1852 µg/ml	2.58	0.70	Ns
Linear relation:	P<0.001		

TABLE 45 : SUMMARY OF THE MUTAGENICITY EXPERIMENT
Experiment with metabolic activation

Test number : 981018
Experiment : Confirmatory
Test substance : CGA 354 743 tech.
Batch : KI-5408/6

Treatment	Mean of viability clones per well	Mean of mutants per flask	Normalized mean of mutants per flask
Negative control	82.83	2.63	3.17
Positive control DMN 1 µl/ml	70.33	164.13	233.35
CGA 354 743 tech.:			
5000.0000 µg/ml	92.42	5.38	5.82
1666.6667 µg/ml	80.92	5.50	6.80
555.5556 µg/ml	79.25	4.13	5.21
185.1852 µg/ml	86.25	4.00	4.64

Treatment	Mean mutant frequency (x10E-6)	Mean mutant factor	Significance (P)
Negative control	1.58		
Positive control DMN 1 µl/ml	116.68	73.64	P<0.001
CGA 354 743 tech.:			
5000.0000 µg/ml	2.91	1.84	0.002<P<0.01
1666.6667 µg/ml	3.40	2.14	0.002<P<0.01
555.5556 µg/ml	2.60	1.64	0.02<P<0.05
185.1852 µg/ml	2.32	1.46	Ns

Linear relation: 0.01<P<0.025

TABLE 6 : SUMMARY OF THE MUTAGENICITY EXPERIMENT
Experiment without metabolic activation

Test number : 981018
Experiment : Confirmatory
Test substance : CGA 354 743 tech.
Batch : KI-5408/6

Treatment	Mean of viability clones per well	Mean of mutants per flask	Normalized mean of mutants per flask
Negative control	62.25	4.13	6.63
Positive control EMS 0.3 µl/ml	59.58	746.13	1252.24
CGA 354 743 tech.:			
5000.0000 µg/ml	65.08	12.13	18.63
1666.6667 µg/ml	62.42	4.63	7.41
555.5556 µg/ml	62.33	7.13	11.43
185.1852 µg/ml	68.42	2.50	3.65

Treatment	Mean mutant frequency (x10E-6)	Mean mutant factor	Significance (P)
Negative control	3.31		
Positive control EMS 0.3 µl/ml	626.12	188.97	P<0.001
CGA 354 743 tech.:			
5000.0000 µg/ml	9.31	2.81	0.001<P<0.002
1666.6667 µg/ml	3.70	1.12	Ns
555.5556 µg/ml	5.72	1.72	0.02<P<0.05
185.1852 µg/ml	1.83	0.55	Ns

Linear relation: P<0.001

TABLE 7 : SUMMARY OF THE MUTAGENICITY EXPERIMENT
Experiment without metabolic activation

Test number : 981018
Experiment : 2nd Confirmatory
Test substance : CGA 354 743 tech.
Batch : KI-5408/6

Treatment	Mean of viability clones per well	Mean of mutants per flask	Normalized mean of mutants per flask
Negative control	53.08	1.25	2.35
Positive control EMS 0.3 µl/ml	37.00	880.13	2378.72
CGA 354 743 tech.:			
5000.0000 µg/ml	51.75	2.75	5.31
1666.6667 µg/ml	52.00	3.25	6.25
555.5556 µg/ml	41.50	3.75	9.04
185.1852 µg/ml	38.42	1.00	2.60

Treatment	Mean mutant frequency (x10E-6)	Mean mutant factor	Significance (P)
Negative control	1.18		
Positive control EMS 0.3 µl/ml	1189.36	1010.16	P<0.001
CGA 354 743 tech.:			
5000.0000 µg/ml	2.66	2.26	Ns
1666.6667 µg/ml	3.13	2.65	0.02<P<0.05
555.5556 µg/ml	4.52	3.84	0.01<P<0.02
185.1852 µg/ml	1.30	1.11	Ns

Linear relation: Ns

DATA EVALUATION RECORD

CGA 51202

STUDY TYPE: MAMMALIAN CELLS IN CULTURE GENE MUTATION ASSAY IN CHINESE HAMSTER V79 CELLS; OPPTS 870.5300 [§84-2]
(MRID 45001201)
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. 01-123

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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.

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Toxicology Branch, Health Effects Division (7509C)

DATA EVALUATION RECORD

STUDY TYPE: Mammalian cells in culture gene mutation assay in Chinese hamster V79 cells [OPPTS 870.5300 (§84-2)]

DP BARCODE: D262423 SUBMISSION CODE: 573839
P.C. CODE: 108801 TOX. CHEM. NO.: none

TEST MATERIAL (PURITY): CGA 51202 tech. (metabolite of CGA 24705) (100% a.i.)

SYNONYMS: none provided

CITATION: Ogorek, B. (1999) CGA-51202 tech. (metabolite of CGA-24705): Final report; Gene mutation test with Chinese hamster cells V79. Genetic Toxicology, Novartis Crop Protection AG, CH-4002 Basel, Switzerland. Laboratory Study ID: 981112, Novartis No. 1192-98, January 18, 1999. MRID 45001201. Unpublished.

SPONSOR: Novartis Crop Protection, Inc., 410 Swing Road, P.O. Box 18300, Greensboro, NC 27419.

EXECUTIVE SUMMARY: In a mammalian cell gene mutation assay at the HPRT locus (MRID 45001201), Chinese hamster V79 cells cultured *in vitro* were exposed to CGA 51202 tech. (Batch No. JD 7069/3, 100% a.i.) in DMSO at concentrations of 500, 1000, 2000 and 4000 µg/mL in the presence and absence of mammalian metabolic activation (S9-mix). A confirmatory assay was conducted at test material concentrations of 375, 750, 1500 and 3000 µg/mL. The S9-fraction was obtained from Aroclor 1254 induced male Tiff:RAI/SPF rat liver.

CGA 51202 tech. was tested up to cytotoxic concentrations. The upper concentrations in both the initial and confirmatory assays, with and without S9-mix, killed virtually all the cells. Statistically significant increases in mean mutant frequency were seen in the initial assay with S9-mix at 500 µg/mL (6.66×10^{-6}) and 1000 µg/mL (5.56×10^{-6}) compared to the solvent control value of 4.02×10^{-6} and without S9-mix at 500 µg/mL (15.35×10^{-6}) compared to the solvent control value of 12.90×10^{-6}. The increases were small and the actual mean mutant frequencies were within the range of historical solvent control values. No positive dose-response was seen and no statistically significant increases in mean mutant frequencies were seen in the confirmatory assay. The solvent and positive controls induced the appropriate response. **There was no evidence of a biologically significant induction of mutant colonies over background.**

This study is classified as **Acceptable/Guideline**. It satisfies the requirement for FIFRA Test Guideline [OPPTS 870.5300 (§84-2)] for *in vitro* mutagenicity (mammalian forward gene mutation) data.

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

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: CGA 51202 tech.

Description: white powder
Lot/Batch #: JD 7069/3
Purity: 100% a.i.
Stability of compound: stable
CAS No.: not provided
Structure: not provided
Solvent used: DMSO
Other comments: none

2. Control materials

Negative: none
Solvent/final concentration: DMSO / 1%
Positive (concentrations/solvent):
Nonactivation: Ethyl methanesulfonate / 0.3 µL/mL / not specified
Activation: N-Nitrosodimethylamine / 1.0 µg/mL / not specified

3. Activation: S9 derived from male Tiff:RAI/SPF rats

x Aroclor 1254	x induced	x rat	x liver
_ phenobarbital	_ non-induced	_ mouse	_ lung
_ none		_ hamster	_ other
_ other		_ other	

If other, describe below

S9 mix composition

S9-fraction	250.0 µL/mL
Glucose-6-phosphate	10.0 µmol/mL
NADP	8.0 µmol/mL
$CaCl_2$	20.0 µmol/mL
$MgCl_2$	20.0 µmol/mL
Na_2HPO_4	1.0 µmol/mL
FCS	30.0 µL/mL

4. Test cells: mammalian cells in culture Chinese hamster V79, clone 65/3 cells

_ mouse lymphoma L5178Y cells
_ Chinese hamster ovary (CHO) cells
x V79 cells (Chinese hamster lung fibroblasts)

Properly maintained? Y
Periodically checked for Mycoplasma contamination? Y
Periodically checked for karyotype stability? Y
Periodically "cleansed" against high spontaneous background? Y

Media: Growth medium was Ham's F10 medium supplemented with 10% fetal calf serum, 100 U/mL penicillin and 100 µg/mL streptomycin. Treatment medium was growth medium with the fetal calf serum reduced to 3% and no antibiotics. Selection medium was growth medium supplemented with 8 µg/mL 6-thioguanine.

5. Locus examined

_ thymidine kinase (TK)
Selection agent: _____ bromodeoxyuridine (BrdU)
_____ fluorodeoxyuridine (FdU)
_____ trifluorothymidine (TFT)

x hypoxanthine-guanine-phosphoribosyl transferase (HPRT)
Selection agent: _____ 8-azaguanine (8-AG)
(give concentr. 8 µg/mL 6-thioguanine (6-TG)

_ Na^+/K^+ ATPase
Selection agent: _____ ouabain
_ other (locus and/or selection agent; give details):

6. Test compound concentrations used

Preliminary cytotoxicity test:
Nonactivated and activated conditions: 2.44, 4.88, 9.77, 19.53, 39.06, 78.12, 156.25, 312.50, 625.00, 1250.00, 2500.00, 5000.00 µg/mL

Mutagenicity assays:

Initial assay:
Nonactivated and activated conditions: 500, 1000, 2000, 4000 µg/mL

Confirmatory assay:
Nonactivated and activated conditions: 375, 750, 1500, 3000 µg/mL

B. TEST PERFORMANCE

1. Cell treatment

a. Cells exposed to test compound, negative/solvent or positive controls for: 21 hours (nonactivated) 5 hours (activated)

b. After washing, cells cultured for 7 - 8 days (expression period) before cell selection:

c. After expression, 2×10^6 cells/dish (4 dishes/ group) were cultured for 7 - 8 days in selection medium to determine numbers of mutants and 100 cells/dish (6 dishes/group) were cultured for 7 - 8 days without selective agent to determine cloning efficiency.

2. Statistical methods: "Statistical significance of mutant frequencies (analysis of variance and test for linear trend) was carried out according to the UKEMS guidelines" (Arlett et al., 1990).

3. Evaluation criteria: Criteria for an acceptable assay were: no effect on results by technical errors, contamination or artifacts; at least three test material concentrations plus the solvent and positive controls should be evaluated; the mutant frequency of the solvent control should not exceed 35×10^{-6}; positive control values should be appropriate and the highest test material concentration should reduce cell viability by 50 - 90% or be limited by solubility or be the limit dose of 5 mg/mL.

The mutant frequency was expressed as mutants per 10^6 survivors and a mean mutant frequency was determined for each dose and activation condition. Cultures with survival values less than 15% were not evaluated for mutagenicity.

Criteria for a positive response were: a valid assay; a mutant frequency at one or more test material concentrations significantly greater than the solvent control with the number of normalized mutant clones in the treated cultures more than 20 greater than the solvent control value; a significant dose-relationship and reproducible results.

II. REPORTED RESULTS

Stock concentrations of test material were analyzed using HPLC with UV detection and found to be about 70% and 79% of the nominal values of 400,000 µg/mL and 50,000 µg/mL, respectively, in the initial assay. Stock concentrations were found to be about 96% and 144% of the nominal values of 300,000 and 37,500 µg/mL, respectively, in the confirmatory assay.

A. PRELIMINARY CYTOTOXICITY ASSAY: Twelve concentrations of CGA 51202 tech. ranging from 2.44 to 5000.00 µg/mL were tested with and without S9-mix in the preliminary cytotoxicity assay. In the presence of S9-mix, almost complete cytotoxicity was seen at 5000 µg/mL, approximately 50% cytotoxicity was seen at concentrations of 625 to 2500 µg/mL, and cytotoxicity ranging from about 14% to 34% was seen at lower concentrations. In the absence of S9-mix, complete cytotoxicity was seen at 5000 µg/mL, approximately 61% cytotoxicity was seen at 2500 µg/mL and cytotoxicity ranging between 7% and 37% seen at lower concentrations. The test material precipitated in culture medium at 5000 µg/mL. Based on these results, an upper concentration of 4000 µg/mL was selected for the initial mutation assay with and without S9-mix. Results of the cytotoxicity test with and without S9-mix are presented in Appendix Tables 1 and 2, respectively (MRID 45001201, pp. 27 and 28).

B. MUTAGENICITY ASSAY

Four concentrations of CGA 51202 tech. ranging from 500 to 4000 µg/mL were tested with and without S9-mix in the initial mutation assay. Two cultures, four flasks per culture, were used at each concentration. No viable cells were seen following the expression period in cultures treated at 4000 µg/mL, either with or without S9-mix. Cell growth was inhibited 22% following treatment at 2000 µg/mL with S9-mix but virtually no cytotoxicity was seen at this dose following the expression period. Cell growth in cultures treated at 2000 µg/mL without S9-mix was inhibited 41% following treatment and 26% after the expression period. The mean mutant frequencies in the presence of S9-mix were 6.66, 5.56 and 4.16 mutants per 10^6 surviving cells at 500, 1000 and 2000 µg/mL, respectively, compared to the solvent control value of 4.02 mutants per 10^6 surviving cells. The increases in mutant frequencies at 500 and 1000 µg/mL were statistically significant ($p < 0.001$) but did not meet the criteria for a positive response and were in the upper range of historical solvent controls. The mean mutant frequencies in the absence of S9-mix were 15.35, 14.46 and 13.18 mutants per 10^6 surviving cells at 500, 1000 and 2000 µg/mL, respectively, compared to the solvent control value of 12.90 mutants per 10^6 surviving cells. The increase in mutant frequency at 500 µg/mL was statistically significant ($0.02 < p < 0.05$) but not considered biologically significant for the same reasons given for the assay with S9-mix. The solvent and control values were acceptable. Results of the initial mutation assay with and without S9-mix are summarized in Appendix Tables 3 and 4, respectively (MRID 45001201, pp. 29 and 30).

A confirmatory mutation assay was conducted using CGA 51202 tech. concentrations of 375, 750, 1500 and 3000 µg/mL with and without S9-mix. Two cultures, four flasks per culture, were used at each concentration. The upper dose, although lower than that used in the initial assay, was excessively cytotoxic both with and without S9-mix, killing virtually all the cells. Cell growth at 1500 µg/mL after treatment and expression was inhibited 48% and 7%, respectively with S9-mix and 57% and 11%, respectively, without S9-mix. The mean mutant frequencies in the presence of S9-mix were 4.33, 2.46, and 3.02 mutants per 10^6 surviving cells at 375, 750 and 1500 µg/mL, respectively, compared to the solvent control value of 3.18 mutants per 10^6 surviving cells. None of the values

were significantly different than the solvent control value. The mean mutant frequencies in the absence of S9-mix were 4.04, 3.96 and 3.24 mutants per 10^6 surviving cells at 375, 750 and 1500 µg/mL, respectively, compared to the solvent control value of 5.74 mutants per 10^6 surviving cells. None of the values were significantly different than the solvent control value. The solvent and positive control values were acceptable. Results of the confirmatory mutation assay with and without S9-mix are summarized in Appendix Tables 5 and 6, respectively (MRID 45001201, pp. 31 and 32).

III. REVIEWER'S DISCUSSION/CONCLUSIONS

A. This is an acceptable study. CGA 51202 tech. was tested to cytotoxic doses, proper experimental protocol was followed and the solvent and positive control values were appropriate. The statistically significant increases in mutant frequency seen in the initial assay are not likely to be biologically significant for a number of reasons. The increases were small, well below the testing laboratory's requirement for a positive response that the number of normalized mutant clones in the treated cultures must be more than 20 greater than the solvent control value. The mutant frequencies were not outside the upper range of historical solvent control frequencies and no positive dose-response was seen. In addition, the results were not confirmed in the repeat assay. There was no evidence that CGA 51202 tech. was mutagenic as tested in this study.

B. STUDY DEFICIENCIES

No major study deficiencies were identified. The concentrations of test material used in the initial assay were 20% to 30% below the desired concentrations but still acceptable. Test material concentrations used in the confirmatory assay were equal to or greater than the desired concentrations.

REFERENCES

Arlett, C.F., D.M. Smith, M.H.L. Green et al. 1990. Mammalian cell gene mutation assays based upon colony formation. In: Statistical Evaluation of Mutagenicity Test Data (ed. Kirkland, D.J.) Cambridge University Press, pp. 66-101.

APPENDIX

(MRID 45001201)

THE FOLLOWING ATTACHMENTS ARE NOT AVAILABLE ELECTRONICALLY. SEE THE FILE COPY.

TABLE 1 : RESULT OF THE CYTOTOXICITY TEST
Experiment with metabolic activation

Test number : 981112
Experiment : Original
Test substance : CGA 51202 tech.
Batch : JD 7069/3

Treatment	Cell number after treatment (x10E6)	Survival clones after treatment (per well)					
Negative control	1.310	82	85	87	80	77	86
Negative control	1.316	81	86	84	78	87	86
CGA 51202 tech.:							
5000.0000 µg/ml	0.259	1	7	3	5	4	5
2500.0000 µg/ml	0.901	61	64	66	60	67	66
1250.0000 µg/ml	0.981	54	60	62	51	55	56
625.0000 µg/ml	0.947	66	61	62	68	64	63
312.5000 µg/ml	1.144	78	82	82	83	84	83
156.2500 µg/ml	1.077	79	89	80	81	80	78
78.1250 µg/ml	0.916	85	81	80	76	78	84
39.0625 µg/ml	0.954	79	81	78	87	81	83
19.5313 µg/ml	0.898	76	82	80	79	83	84
9.7656 µg/ml	1.094	81	86	80	77	84	84
4.8828 µg/ml	1.115	85	80	81	78	79	86
2.4414 µg/ml	1.019	77	86	86	83	81	80

Treatment	Mean of clones	Number of viable cells (x10E6)	Acute cytotoxicity (% of control)
Negative control	82.83	1.09	
Negative control	83.67	1.10	
CGA 51202 tech.:			
5000.0000 µg/ml	4.17	0.01	99.01
2500.0000 µg/ml	64.00	0.58	47.25
1250.0000 µg/ml	56.33	0.55	49.47
625.0000 µg/ml	64.00	0.61	44.56
312.5000 µg/ml	82.00	0.94	14.20
156.2500 µg/ml	81.17	0.87	20.03
78.1250 µg/ml	80.67	0.74	32.38
39.0625 µg/ml	81.50	0.78	28.91
19.5313 µg/ml	80.67	0.72	33.77
9.7656 µg/ml	82.00	0.90	17.91
4.8828 µg/ml	81.50	0.91	16.89
2.4414 µg/ml	82.17	0.84	23.38

TABLE 2 : RESULT OF THE CYTOTOXICITY TEST
Experiment without metabolic activation

Test number : 981112
Experiment : Original
Test substance : CGA 51202 tech.
Batch : JD 7069/3

Treatment	Cell number after treatment (x10E6)	Survival clones after treatment (per well)					
Negative control	1.094	96	100	96	91	101	92
Negative control	1.388	92	98	96	89	96	99
CGA 51202 tech.:							
5000.0000 µg/ml	Tx	*	*	*	*	*	*
2500.0000 µg/ml	0.512	96	90	86	84	90	96
1250.0000 µg/ml	1.138	95	88	91	94	87	91
625.0000 µg/ml	1.028	92	96	89	97	104	100
312.5000 µg/ml	1.003	101	96	92	93	98	97
156.2500 µg/ml	1.080	89	90	95	95	98	99
78.1250 µg/ml	0.940	89	88	96	96	105	97
39.0625 µg/ml	0.784	92	93	96	100	99	90
19.5313 µg/ml	0.871	91	98	92	93	104	98
9.7656 µg/ml	0.977	96	99	100	95	92	97
4.8828 µg/ml	1.245	87	97	98	104	96	92
2.4414 µg/ml	1.141	101	96	92	97	94	99

Treatment	Mean of clones	Number of viable cells (x10E6)	Acute cytotoxicity (% of control)
Negative control	96.00	1.05	
Negative control	95.00	1.32	
CGA 51202 tech.:			
5000.0000 µg/ml	*	*	*
2500.0000 µg/ml	90.33	0.46	60.96
1250.0000 µg/ml	91.00	1.04	12.61
625.0000 µg/ml	96.33	0.99	16.40
312.5000 µg/ml	96.17	0.96	18.61
156.2500 µg/ml	94.33	1.02	13.99
78.1250 µg/ml	95.17	0.89	24.46
39.0625 µg/ml	95.00	0.75	37.10
19.5313 µg/ml	96.00	0.84	29.46
9.7656 µg/ml	96.50	0.94	20.45
4.8828 µg/ml	95.67	1.19	nTx
2.4414 µg/ml	96.50	1.10	7.03

TABLE 3 : SUMMARY OF THE MUTAGENICITY EXPERIMENT
Experiment with metabolic activation

Test number : 981112
Experiment : Original
Test substance : CGA 51202 tech.
Batch : JD 7069/3

Treatment	Mean of viability clones per well	Mean of mutants per flask	Normalized mean of mutants per flask
Negative control	96.50	7.75	8.03
Positive control DMN 1 µl/ml	82.58	137.00	165.89
CGA 51202 tech.:			
4000.0000 µg/ml	*	*	*
2000.0000 µg/ml	96.08	8.00	8.33
1000.0000 µg/ml	93.25	10.38	11.13
500.0000 µg/ml	97.67	13.00	13.31

Treatment	Mean mutant frequency (x10E-6)	Mean mutant factor	Significance (P)
Negative control	4.02		
Positive control DMN 1 µl/ml	82.95	20.66	P<0.001
CGA 51202 tech.:			
4000.0000 µg/ml	*	*	*
2000.0000 µg/ml	4.16	1.04	Ns
1000.0000 µg/ml	5.56	1.39	P<0.001
500.0000 µg/ml	6.66	1.66	P<0.001

Linear relation: Ns

TABLE 4 : SUMMARY OF THE MUTAGENICITY EXPERIMENT
Experiment without metabolic activation

Test number : 981112
Experiment : Original
Test substance : CGA 51202 tech.
Batch : JD 7069/3

Treatment	Mean of viability clones per well	Mean of mutants per flask	Normalized mean of mutants per flask
Negative control	91.08	23.50	25.80
Positive control EMS 0.3 µl/ml	60.42	775.50	1283.59
CGA 51202 tech.:			
4000.0000 µg/ml	*	*	*
2000.0000 µg/ml	59.75	15.75	26.36
1000.0000 µg/ml	64.42	18.63	28.91
500.0000 µg/ml	68.42	21.00	30.69

Treatment	Mean mutant frequency (x10E-6)	Mean mutant factor	Significance (P)
Negative control	12.90		
Positive control EMS 0.3 µl/ml	641.79	49.75	P<0.001
CGA 51202 tech.:			
4000.0000 µg/ml	*	*	*
2000.0000 µg/ml	13.18	1.02	Ns
1000.0000 µg/ml	14.46	1.12	Ns
500.0000 µg/ml	15.35	1.19	0.02<P<0.05

Linear relation: Ns

TABLE 5 : SUMMARY OF THE MUTAGENICITY EXPERIMENT
Experiment with metabolic activation

Test number : 981112
Experiment : Confirmatory
Test substance : CGA 51202 tech.
Batch : JD 7069/3

Treatment	Mean of viability clones per well	Mean of mutants per flask	Normalized mean of mutants per flask
Negative control	80.67	5.13	6.35
Positive control DMN 1 µl/ml	57.00	103.75	182.02
CGA 51202 tech.:			
3000.0000 µg/ml	*	*	*
1500.0000 µg/ml	80.67	4.88	6.04
750.0000 µg/ml	81.25	4.00	4.92
375.0000 µg/ml	83.75	7.25	8.66

Treatment	Mean mutant frequency (x10E-6)	Mean mutant factor	Significance (P)
Negative control	3.18		
Positive control DMN 1 µl/ml	91.01	28.65	P<0.001
CGA 51202 tech.:			
3000.0000 µg/ml	*	*	*
1500.0000 µg/ml	3.02	0.95	Ns
750.0000 µg/ml	2.46	0.77	Ns
375.0000 µg/ml	4.33	1.36	Ns

Linear relation: Ns

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TABLE 6 : SUMMARY OF THE MUTAGENICITY EXPERIMENT
Experiment without metabolic activation

Test number : 981112
Experiment : Confirmatory
Test substance : CGA 51202 tech.
Batch : JD 7069/3

Treatment	Mean of viability clones per well	Mean of mutants per flask	Normalized mean of mutants per flask
Negative control	71.92	8.25	11.47
Positive control EMS 0.3 µl/ml	46.58	374.50	803.94
CGA 51202 tech.:			
3000.0000 µg/ml	*	*	*
1500.0000 µg/ml	75.17	4.88	6.49
750.0000 µg/ml	71.00	5.63	7.92
375.0000 µg/ml	77.33	6.25	8.08

Treatment	Mean mutant frequency (x10E-6)	Mean mutant factor	Significance (P)
Negative control	5.74		
Positive control EMS 0.3 µl/ml	401.97	70.08	P<0.001
CGA 51202 tech.:			
3000.0000 µg/ml	*	*	*
1500.0000 µg/ml	3.24	0.57	Ns
750.0000 µg/ml	3.96	0.69	Ns
375.0000 µg/ml	4.04	0.70	Ns

Linear relation: 0.025<P<0.05

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Supplement to Document #001374 - DER for MRID No.00080897: Multi-generation Reproduction Study in Rats. This supplement provides an Executive Summary to upgrade the original DER.

EPA Reviewer: Virginia A. Dobozy, V.M.D., M.P.H. Virginia A Dobozy 7/10/01
Reregistration Branch I, Health Effects Division (7509C)

Branch Senior Scientist: Whang Phang, Ph.D. Wh Ph 7/26/01
Reregistration Branch I, Health Effects Division (7509C)

DATA EVALUATION RECORD

STUDY TYPE: Multi-generation Reproduction Study in Rats
OPPTS Number: 870.3800 OPP Guideline Number: 83-4

PC CODE: 108801

TEST MATERIAL (PURITY): Metolachlor (95.4% a.i.)

Chemical Name: 2-chloro-N-(2-ethyl-6-methylphenyl)-N-(2-methoxy-1-methylethyl) acetamide

Synonym: CGA-24705

CITATION: Page, J.G. (1981) Two-Generation Reproduction Study in Albino Rats with Metolachlor Technical. Toxigenics, Decatur, IL. Study Number 450-0272, August 31, 1981. MRID No. 00080897. Unpublished.

SPONSOR: CIBA-GEIGY Corporation

EXECUTIVE SUMMARY:

In a two-generation reproduction study (MRID 00080897), metolachlor (95.4% a.i.) was administered in the diet to two consecutive generations of 15 male/30 female CD albino rats at dose levels of 0, 30, 300 or 1000 ppm (F_0 males: 0, 2.4, 23.5 and 75.8 mg/kg/day; F_0 females: 0, 2.5, 26.0 and 85.7 mg/kg/day; F_1males: 0, 2.3, 23.7 and 76.6 mg/kg/day; F_1 females: 0, 2.6, 25.7 and 84.5 mg/kg/day).

There were no deaths in the F_0 generation. Two females of the F_1 generation died during the pre-mating period, one in the 300 ppm group at 32 days and the other in the 1000 ppm group at 52

days. One female in the 300 ppm group was found dead on gestation day 19 and a control group female was sacrificed in a moribund condition on lactation day 1. Based on necropsy examinations, none of the deaths was treatment-related. There were no treatment-related clinical signs of toxicity in either generation. Body weight, body weight gain and food consumption were unaffected in the F_0 generation. In the F_1 generation, food consumption was significantly decreased in females of the 1000 ppm group at several timepoints; however, there was no effect on body weight/body weight gain. Therefore, this finding was not considered toxicologically significant. There were no treatment-related effects on organ weights or gross/microscopic necropsy examinations in either generation.

There was no evidence of a treatment-related effect on any of the reproductive parameters for either generation. Offspring body weight was significantly decreased in the F_1 litter on lactation days 14 and 21 (91- 96% of control value) and in the F_2 litter on lactation days 4, 7, 14 and 21 (92 - 95% of control value). Although the magnitude of the decrease is small, the finding is regarded as toxicologically significant.

The parental toxicity LOAEL was not established. The NOAEL was 1000 ppm (F_0 males/females: 75.8/85.7 mg/kg/day; F_1males/females: 76.6/84.5 mg/kg/day).

The reproductive toxicity LOAEL was not established. The NOAEL was 1000 ppm (F_0 males/females: 75.8/85.7 mg/kg/day; F_1males/females: 76.6/84.5 mg/kg/day).

The offspring LOAEL was conservatively established at 1000 ppm (F_0 males/females: 75.8/85.7 mg/kg/day; F_1males/females: 76.6/84.5 mg/kg/day) based on decreased body weight in F_1 and F_2 litters . The NOAEL is 300 ppm (F_0 males/females: 23.5/ 26.0 mg/kg/day; F_1males/females: 23.7/25.7 mg/kg/day).

The study is classified as **Acceptable/guideline** and **satisfies** the guideline requirements for a multi-generation reproduction study in rats (83-4; OPPTS 870.3800).

Supplement to Document #010251- DER for MRID 00129377: Chronic Toxicity/Carcinogenicity Study in Rats. This supplement provides an Executive Summary to upgrade the original DER which reviewed only the chronic toxicity portion of the study.

EPA Reviewer: Virginia A. Dobozy, V.M.D., M.P.H. Virginia A Dobozy 7/11/01
Reregistration Branch I, Health Effects Division (7509C)

Branch Senior Scientist: Whang Phang, Ph.D. Whang Phang 07/25/01
Reregistration Branch I, Health Effects Division (7509C)

DATA EVALUATION RECORD

STUDY TYPE: Chronic Toxicity/Carcinogenicity Study in Rats

OPPTS Number: 870.4100 OPP Guideline Number: 83-1

PC CODE: 108801

TEST MATERIAL (PURITY): Metolachlor (95.3% a.i.)

Chemical Name: 2-chloro-N-(2-ethyl-6-methylphenyl)-N-(2-methoxy-1-methylethyl) acetamide

Synonym: CGA-24705

CITATION: Tisdel, M. (1983) Two-year Chronic Oral Toxicity and Oncogenicity Study with Metolachlor in Albino Rats. Hazleton-Raltech, Inc., Madison, WI. Study Number 80030, May 2, 1983. MRID No. 00129377. Unpublished.

SPONSOR: CIBA-GEIGY Corporation

EXECUTIVE SUMMARY:

In a chronic toxicity/carcinogenicity study (MRID 00129377), metolachlor (95.3% a.i.) was administered in the diet to 60 CD-Crl:CD (SD)BR albino rats/sex/group at dose levels of 0, 30, 300 or 3000 ppm (0, 1.5, 15 or 150 mg/kg/day based on 1 ppm in food equals 0.05 mg/kg/day) for two years. An additional 10 rats/sex/group were administered either 0 (control) or 3000 ppm in the diet for 12 months; five rats/sex/group were sacrificed after the treatment and the remaining five/sex/group were allowed to recover for four weeks and then sacrificed.

This summary applies only to the chronic toxicity portion of the study. The HED Cancer Peer

Review Committee evaluated the carcinogenic potential of metolachlor on several occasions. At the 4th Committee meeting on July 27, 1994, metolachlor was classified as a Group C, possible human carcinogen based on liver tumors in rats with risk quantitated using a Margin of Exposure approach.

Comparable mortality rates were observed in the treated and control animals. There were no treatment-related clinical signs of toxicity. Mean body weight gain was slightly decreased in the 3000 ppm females (6 - 17% decrease) throughout the study; the changes were not statistically significant. Mean food consumption was slightly decreased (4 - 9%) in the 3000 ppm females; the decrease was not statistically significant. Absolute, relative and liver-to-brain weight were increased (7%, 13% and 5%, respectively) in the 3000 ppm males. These increases were also observed in the 3000 ppm males after the four-week recovery period. However, the toxicological significance of the finding is questionable as there were no accompanying clinical pathology or histological changes.

The LOAEL was 3000 ppm (150 mg/kg/day) for females based on slightly decreased body weight gain and food consumption. The NOAEL was 300 ppm (15 mg/kg/day) for females. The LOAEL was not established for males. The NOAEL was 3000 ppm (150 mg/kg/day).

The study is classified as **acceptable/guideline** and **satisfies** the guideline requirements for a chronic toxicity study in rats (83-1; OPPTS 870.4100).

Supplement to Document #003885 - DER for MRID 00117597: Carcinogenicity Study in Mice. This supplement provides an Executive Summary and an additional data table to upgrade the original DER.

EPA Reviewer: Virginia A. Dobozy, V.M.D., M.P.H. Virginia A Dobozy 7/31/01
Reregistration Branch I, Health Effects Division (7509C)

Branch Senior Scientist: Whang Phang, Ph.D. Whang Phang 7/31/01
Reregistration Branch I, Health Effects Division (7509C)

DATA EVALUATION RECORD

STUDY TYPE: Carcinogenicity Study in Mice

OPPTS Number: 870.4200 OPP Guideline Number: 83-5

PC CODE: 108801

TEST MATERIAL (PURITY): Metolachlor (reported to be 95% a.i.)

Chemical Name: 2-chloro-N-(2-ethyl-6-methylphenyl)-N-(2-methoxy-1-methylethyl) acetamide

Synonym: CGA-24705

CITATION: Tisdel, M. (1982) Carcinogenicity Study with Metolachlor in Albino Mice. Hazleton-Raltech, Inc., Madison, WI. Study Number 79020, August 13, 1982. MRID No. 00117597. Unpublished.

SPONSOR: CIBA-GEIGY Corporation

EXECUTIVE SUMMARY:

In a carcinogenicity study (MRID 00117597), metolachlor (reported to be 95% a.i.) was administered in the diet to 68 CD-1 mice/sex/group at doses of 0, 300, 1000 or 3000 ppm (0, 45, 150 or 450 mg/kg/day, based on 1ppm equals 0.150 mg/kg/day). Eight mice/sex/group were sacrificed at 12 and 18 months.

High dose females had a significant increased mortality rate due to a number of deaths during the first few weeks of treatment (control: 24/52; high dose females: 34/52 at termination) . Although

the deaths were possibly attributable to a viral infection, the contribution of the test material can't be dismissed. Body weight was statistically significantly decreased (91-95% of control value) throughout the study in the 3000 ppm males and during the latter half of the study in the 3000 ppm females (93-95%). Body weight gain was consistently decreased in the 3000 ppm males (48-88%) and females (59-86%). Food consumption was comparable between treated and control groups until week 90 of treatment, at which time the 3000 ppm males consumed 10% less than controls. The decrease was statistically significant at weeks 98, 102 and 104. There was no significant effect on female food consumption. There was no evidence of a treatment-related effect on hematology or clinical chemistry parameters. Organ weight was not affected except for a dose-related decrease in the absolute and relative weight of the seminal vesicles of males which was statistically significant at the high dose. However, there was no effect on testes weight and no accompanying histological changes in the seminal vesicles; therefore, the toxicological significance of the finding is questionable. There were no treatment-related microscopic changes. There was no treatment-related increase in tumor incidence in the study.

The LOAEL was 3000 ppm (450 mg/kg/day) based on possible treatment-related deaths in females and decreased body weight/body weight gain in males and females. The NOAEL was 1000 ppm (150 mg/kg/day).

The HED Cancer Peer Review Committee evaluated the carcinogenic potential of metolachlor on several occasions. At the 4th Committee meeting on July 27, 1994, it was metolachlor was classified as a Group C, possible human carcinogen based on liver tumors in the rat with risk quantitated using a Margin of Exposure approach.

The study is classified as **acceptable/guideline** and **satisfies** the guideline requirements for a carcinogenicity toxicity study in mice (83-5; OPPTS 870.4200).

Table 1: Body Weight/Body Weight Gain in Mice Treated with Metolachlor[a]

	Dose Levels (ppm)							
	Males				Females			
Week	0	300	1000	3000	0	300	1000	3000
Mean Body Weight (g)[a]								
0	23.1	23.5	23.2	23.1	19.3	19.2	19.4	19.7
1	24.0	24.3	23.5	23.1	19.2	19.1	19.0	19.0
2	26.6	26.5	26.0	24.8** (93)	21.5	21.1	21.3	21.0
13	33.6	33.3	33.3	31.9** (95)	26.6	26.6	27.2	26.0
26	36.9	37.3	36.8	34.8** (95)	29.6	29.3	29.9	28.9
52	40.1	39.8	39.3	36.4** (91)	32.0	32.0	31.9	30.4* (95)
104	40.5	40.9	39.7	37.9* (94)	35.2	34.3	34.7	32.6 (93)
Weeks	Body Weight Gain (g)							
0-1	0.9	0.8	0.7	0	-0.1	-0.1	-0.4	-0.7
0-2	3.5	3.0	2.8 (80)	1.7 (48)	2.2	1.9	1.9	1.3 (59)
0-13	10.5	9.8	10.1	8.8 (84)	7.3	7.4	7.8	6.3 (86)
13-26	3.3	4.0	3.5	2.9 (88)	3.0	2.7	2.7	2.9
13-52	6.5	6.5	6.0	4.5 (69)	5.4	5.4	4.7	4.4 (81)
0-52	17.0	16.3	16.1	13.3 (78)	12.7	12.8	12.5	10.7 (84)
0-104	17.4	17.4	16.5	14.8 (85)	15.9	15.1	15.3	12.9 (81)

a Extracted from Tables 20 (pages 83-84) and 23 (pages 91-92) of the study report.
b Calculated by the reviewer using mean body weight data
* $p<0.05$ and ** $p<0.01$ using Dunnett's procedure
(percentage of control value, calculated by reviewer)

Supplement to Documents # 010251, 010088, 009699 and 008442- DER for MRID Nos. 40980701, 41164501, 42218601 and 42218602: Chronic Oral Toxicity Study in Dogs. This supplement provides an Executive Summary to upgrade the original DERs.

EPA Reviewer: Virginia A. Dobozy, V.M.D., M.P.H. Virginia A Dobozy 7/10/01
Reregistration Branch I, Health Effects Division (7509C)

Branch Senior Scientist: Whang Phang, Ph.D. 7/26/01
Reregistration Branch I, Health Effects Division (7509C)

DATA EVALUATION RECORD

STUDY TYPE: Chronic Oral Toxicity Study in Dogs
OPPTS Number: 870. 4100 OPP Guideline Number: 83-1

PC CODE: 108801

TEST MATERIAL (PURITY): Metolachlor (97% a.i.)

Chemical Name: 2-chloro-N-(2-ethyl-6-methylphenyl)-N-(2-methoxy-1-methylethyl) acetamide

Synonym: CGA-24705

CITATION: Hazellette, J.R. and Arthur, A. T. (1989) Metolachlor Technical, 13/52-Week Oral Toxicity Study in Dogs (MIN 862253). Division of Toxicology/Pathology, CIBA-GEIGY, Summit, NJ and Methpath Laboratories, Rockville, MD, Study Number 862253, January 23, 1989. MRID Nos. 40980701, 41164501, 42218601 and 42218602. Unpublished.

SPONSOR: CIBA-GEIGY Corporation

EXECUTIVE SUMMARY:

In a chronic toxicity study (MRIDs 40980701, 41164501, 42218601 and 42218602), metolachlor (97% a.i.) was administered in the diet to Beagle dogs (6/sex/group for control and high dose groups; 4/sex/group for low- and mid-dose groups) at dose levels of 0, 100, 300 or 1000 ppm (males: 0, 3.5, 9.7 and 32.7 mg/kg/day, respectively; females: 0, 3.6, 9.7 and 33.0 mg/kg/day, respectively) for one year. Two dogs of each sex in the control and high-dose group designated as recovery animals were treated for 52 weeks and were then allowed a 4-week recovery period. An additional 4 dogs/sex/group were treated at the same dose levels and

Supplement to Document #009509 - DER for MRID No.00151941: Prenatal Developmental Toxicity Study in Rats. This supplement provides an Executive Summary to upgrade the original DER.

EPA Reviewer: Virginia A. Dobozy, V.M.D., M.P.H. Virginia A Dobozy 7/10/01
Reregistration Branch I, Health Effects Division (7509C)

Branch Senior Scientist: Whang Phang, Ph.D. Wh Phang 7/26/01
Reregistration Branch I, Health Effects Division (7509C)

DATA EVALUATION RECORD

STUDY TYPE: Prenatal Developmental Toxicity - Rat
OPPTS Number: 870.3700 OPP Guideline Number: 83-3a

PC CODE: 108801

TEST MATERIAL (PURITY): CGA-24705 (Metolachlor) (96.4% a.i.)

Chemical Name: 2-chloro-N-(2-ethyl-6-methylphenyl)-N-(2-methoxy-1-methylethyl) acetamide

CITATION: Lochry, E.A. (1985) Embryo/Fetal Toxicity and Teratogenic Potential Study of CGA-24705 (FL-841697) Administered Orally via Gavage to Crl:COBS®CD®(SD) BR Presumed Pregnant Rats. Argus Research Laboratories, Inc., Horsham, PA. Argus Project 203-004, August 6, 1985. MRID No. 00151941. Unpublished.

SPONSOR: CIBA-GEIGY Corporation

EXECUTIVE SUMMARY:

In a prenatal developmental toxicity study (MRID 00151941), CGA-24705 (metolachlor) (96.4% a.i.) in 0.5% (w/v) aqueous hydroxymethylcellulose was administered by gavage (10 ml/kg) to 25 presumed pregnant Crl:COBS®CD®(SD) BR rats from gestation days (GD) 6 through 15, inclusive, at dose levels of 0, 30, 100, 300 or 1000 mg/kg/day. The animals were sacrificed on GD 20 and the fetuses examined for evidence of developmental effects.

There were four treatment-related deaths [GD 7, 8 and 10 (2 rats)] in animals treated at 1000 mg/kg/day. Clinical signs of toxicity, including clonic and/or toxic convulsions, excessive salivation, urine-stained abdominal fur and/or excessive lacrimation, were observed in animals treated at 1000 mg/kg/day. There was also an increase in excessive salivation in the 300 mg/kg/day group. However, as this effect was most likely due to gastric irritation and there was

no other evidence of treatment-related toxicity, the finding is not considered toxicologically significant. Body weight gain was significantly decreased in the 1000 mg/kg/day group during GD 6-16 (83% of control value; $p<0.05$), GD 6-20 (88% of control value; $p<0.05$) and GD 0-20 (88% of control value; $p<0.01$). Food consumption was not affected.

In the 1000 mg/kg/day group, there was a slightly decreased number of implantations per dam (14.6 vs 15.8 in controls), decreased live fetuses/dam (13.8 vs 15.2 in controls) and increased number of resorptions/dam (0.8 vs 0.5 in controls). There was also a statistically significant decrease ($p<0.05$; 96% of control value) in mean fetal body weight.

The maternal toxicity LOAEL was 1000 mg/kg/day based on an increased incidence of death, clinical signs of toxicity (clonic and/or toxic convulsions, excessive salivation, urine-stained abdominal fur and/or excessive lacrimation) and decreased body weight gain. The NOAEL was 300 mg/kg/day.

The developmental toxicity LOAEL was conservatively established at 1000 mg/kg/day based on slightly decreased number of implantations per dam, decreased number of live fetuses/dam, increased number of resorptions/dam and significant decrease in mean fetal body weight. The NOAEL was 300 mg/kg/day.

The study is classified as **Acceptable/guideline** and **satisfies** the guideline requirements for a prenatal developmental toxicity study in rats (83-3a; OPPTS 870.3700).

Supplement to Document #001051- DER for MRID No.00041283: Prenatal Developmental Toxicity Study in Rabbits. This supplement provides an Executive Summary to upgrade the original DER.

EPA Reviewer: Virginia A. Dobozy, V.M.D., M.P.H. Virginia A Dobozy 7/10/01
Reregistration Branch I, Health Effects Division (7509C)

Branch Senior Scientist: Whang Phang, Ph.D. Whang Phang 7/26/01
Reregistration Branch I, Health Effects Division (7509C)

DATA EVALUATION RECORD

STUDY TYPE: Prenatal Developmental Toxicity - Rabbit
OPPTS Number: 870. 3700 OPP Guideline Number: 83-3b

PC CODE: 108801

TEST MATERIAL (PURITY): CGA-24705 (Metolachlor) (95.4% a.i.)

Chemical Name: 2-chloro-N-(2-ethyl-6-methylphenyl)-N-(2-methoxy-1-methylethyl) acetamide

CITATION: Lightkep, G.E. (1980) Teratogenic Potential of CGA-24705 in New Zealand White Rabbits Segment II Evaluation. Argus Research Laboratories, Inc., Horsham, PA. Argus Project 203-001, July 16, 1980. MRID No. 00041283. Unpublished.

SPONSOR: CIBA-GEIGY Corporation

EXECUTIVE SUMMARY:

In a prenatal developmental toxicity study (MRID 00041283), CGA-24705 (metolachlor) (95.4% a.i.) in 0.75% aqueous hydroxy methylcellulose was administered by gavage (10 ml/kg) to 16 pregnant New Zealand White rabbits/group from gestation days (GD) 6 through 18, inclusive, at dose levels of 0, 36, 120 or 360 mg/kg/day. The animals were sacrificed on GD 30 and the fetuses examined for evidence of developmental effects.

One doe at 36 mg/kg/day and another at 360 mg/kg/day died on GDs 24 and 29, respectively. The cause of death in both animals was attributed to persistent anorexia. Two rabbits aborted, one at 120 mg/kg/day (GD 25) and another at 360 mg/kg/day (GD 17). The high-dose animal had persistent anorexia. One rabbit in each group delivered prior to GD 30; the control, low- and

high-dose animals on GD 29 and the mid-dose animal on GD 30. There was a treatment-related increase in the incidence of persistent anorexia in the does treated at 360 mg/kg/day, which was defined as less than one-half of the daily food allotment consumed. However, food consumption data were not provided to support this finding. There was a treatment-related decrease in body weight gain in the 360 mg/kg/day group for GD 6-18 (-0.16 kg vs +0.04 kg in controls; $p<0.01$) and GD 6-30 (-0.01kg vs +0.03 kg in controls). There was no treatment-related increase in gross pathological findings in maternal animals at necropsy.

No treatment-related increase in external, visceral or skeletal developmental effects was observed.

The maternal toxicity LOAEL was 360 mg/kg/day based on an increased incidence of clinical observations (persistent anorexia) and decreased body weight gain. The NOAEL was 120 mg/kg/day.

The developmental toxicity LOAEL was not established. The NOAEL was 360 mg/kg/day.

The study is classified as **Acceptable/guideline** and **satisfies** the guideline requirements for a prenatal developmental toxicity study in rabbits (83-3b; OPPTS 870.3700).

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Supplement to Documents #010251and 011115 - DER for MRID Nos.00032174 and 43244001: Subchronic Oral Toxicity Study in Dogs. This supplement provides an Executive Summary to upgrade the original DER.

EPA Reviewer: Virginia A. Dobozy, V.M.D., M.P.H. Virginia A Dobozy 9/5/01
Reregistration Branch I, Health Effects Division (7509C)

Branch Senior Scientist: Whang Phang, Ph.D. Whang Phang 09/05/01
Reregistration Branch I, Health Effects Division (7509C)

DATA EVALUATION RECORD

STUDY TYPE: Subchronic Oral Toxicity Study in Dogs
OPPTS Number: 870.3150 OPP Guideline Number: 82-1

PC CODE: 108801

TEST MATERIAL (PURITY): Metolachlor

Chemical Name: 2-chloro-N-(2-ethyl-6-methylphenyl)-N-(2-methoxy-1-methylethyl) acetamide

Synonym: CGA-24705

CITATION: Estes, F.L. (1980) 6-Month Chronic Oral Toxicity Study in Beagle Dogs. International Research and Development Corporation (IRDC), Mattawan, MI., Study Number 382-054, May 21, 1980. MRID No. 00032174. Unpublished.

SPONSOR: CIBA-GEIGY Corporation

EXECUTIVE SUMMARY:

In a subchronic oral toxicity study (MRIDs 00032174 and 43244001), metolachlor (96.8% ai) was administered in the diet to Beagle dogs (8/sex/group for control and high dose groups; 6/sex/group for low- and mid-dose groups) at dose levels of 0, 100, 300 or 1000 ppm (males: 0, 2.92, 9.71 and 29.61 mg/kg/day, respectively; females: 0, 2.97, 8.77 and 29.42 mg/kg/day, respectively) for six months.

There were no deaths or clinical signs of toxicity. Mean body weight gain was decreased during weeks 0-13 and 0-26 in the 1000 ppm group males (55-63% decrease) and females (44-50%

decrease), although the changes were not statistically significant. Mean overall food consumption was not affected in the 1000 ppm group males but was slightly decreased (9%) in the 1000 ppm females. There was a significant decrease in the activated partial thromboplastin time (APTT) in the 300 and 1000 ppm group males and 300 ppm group females but the findings were not considered toxicologically significant because the decrease was slight and not dose-related. Alkaline phosphatase was signifcantly increased in the 300 ppm and 1000 ppm group males and females at week 26; however, the effect was not considered toxicologically significant due to the small magnitude of the increase and the lack of accompanying necropsy findings.

The LOAEL was 1000 ppm (males/females: 29.61/29.42 mg/kg/day) based on decreased body weight gain. The NOAEL was 300 ppm (males/females: 9.71/8.77 mg/kg/day).

The study is classified as **Acceptable/guideline** and **satisfies** the guideline requirements for a subchronic toxicity study in dogs (82-1; OPPTS 870.3150). The study was conducted for six months, whereas the guidelines require 90 days of dosing. However, toxicity parameters, with the exception of necropsy, were also evaluated at 90 days in the study.

Supplement to Documents #009558 and 010315 - DER for MRID No.41833101: 21-Day Dermal Toxicity Study in Rabbits. This supplement provides an Executive Summary to upgrade the original DER. The systemic NOAEL/LOAEL have been changed on re-evaluation.

EPA Reviewer: Virginia A. Dobozy, V.M.D., M.P.H. Virginia A Dobozy 7/10/01
Reregistration Branch I, Health Effects Division (7509C)

Branch Senior Scientist: Whang Phang, Ph.D. 07/25/01
Reregistration Branch I, Health Effects Division (7509C)

DATA EVALUATION RECORD

STUDY TYPE: 21-Day Dermal Toxicity Study in Rabbits
OPPTS Number: 870.3200 OPP Guideline Number: 82-2

PC CODE: 108801

TEST MATERIAL (PURITY): Metolachlor (96.4% a.i.)

Chemical Name: 2-chloro-N-(2-ethyl-6-methylphenyl)-N-(2-methoxy-1-methylethyl) acetamide

Synonym: CGA-24705

CITATION: Mastrocco, F., Huber, K., Schiavo, D.M., Hazelette, J.R. and Green, J.D. 21-Day Dermal Toxicity Study in Rabbits. Research Department, Pharmaceuticals Division, CIBA-GEIGY Corporation. Study Number 862012, November 16, 1987. MRID No. 41833101. Unpublished.

SPONSOR: CIBA-GEIGY Corporation

EXECUTIVE SUMMARY:

In a 21-day dermal toxicity study (MRID 41833101), metolachlor (96.4% a.i.) was applied topically once daily for 21 days to the intact skin of five New Zealand rabbits/sex/group at doses of 0, 10, 100 or 1000 mg/kg/day.

All animals survived the treatment. There were no treatment-related effects on clinical signs, body weight/body weight gain, food consumption, ophthalmoscopic examinations, hematology

or necropsy examinations. Significant increases in total bilirubin were observed only in females treated at 100 mg/kg/day (68% increase) and 1000 mg/kg/day (72% increase). However, these increases were not considered toxicologically significant as there was no other evidence of organ effects at these doses and hyperbilirubinemia has not been reported in other toxicity studies with metolachlor. Absolute and relative liver weight were significantly increased in the 1000 mg/kg/day males and relative kidney weight was significantly increased in 1000 mg/kg/day females. These effects are not considered toxicologically significant as there were no accompanying laboratory or necropsy findings.

There was evidence of skin irritation in all treated groups. Very slight erythema and dry skin were observed in all animals of the 10 mg/kg/day group; one female at this dose had fissuring. With increasing doses, more animals were observed to have fissuring and wrinkling of the skin. On histopathology, hyperkeratosis, parakeratosis, congestion of the dermis, edema and subacute lymphocytic infiltration were reported in some or all of the treated animals.

The systemic LOAEL was not established. The NOAEL was 1000 mg/kg/day (HDT).

The dermal irritation LOAEL was 10 mg/kg/day (LDT) based on very slight erythema, dry skin and fissuring (one animal). The NOAEL was not established.

The study is classified as **acceptable/guideline** and **satisfies** the guideline requirements for a 21-day dermal toxicity study in rabbits (82-2; OPPTS 870.3200).

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Supplement to Document # 009558- DER for MRID No.41833102: Dermal Penetration Study in Rats. This supplement provides an Executive Summary to upgrade the original DER.

EPA Reviewer: Virginia A. Dobozy, V.M.D., M.P.H. Virginia A Dobozy 8/14/01
Reregistration Branch I, Health Effects Division (7509C)

Branch Senior Scientist: Whang Phang, Ph.D. Whang Phang 8/14/01
Reregistration Branch I, Health Effects Division (7509C)

DATA EVALUATION RECORD

STUDY TYPE: Dermal Penetration in Rats

OPPTS Number: 870.7600 OPP Guideline Number: 85-3

PC CODE: 108801

TEST MATERIAL (PURITY): ^{14}C-Metolachlor (uniformly labeled in the phenyl with a specific activity of 17.7 µCi/ng for the low- and mid-dose levels and 1.77 µg/mg for the high-dose level)

Chemical Name: 2-chloro-N-(2-ethyl-6-methylphenyl)-N-(2-methoxy-1-methylethyl) acetamide

Synonym: CGA-24705

CITATION: Murphy, T. (1987). Dermal Absorption of Metolachlor in Rats. CIBA-GEIGY Corporation, Agricultural Division, Metabolism Department, Greensboro, NC and WIL Research Laboratories, Ashland, Ohio. Laboratory Study No. ABR-87051, August 25, 1987. MRID No. 41833102. Unpublished.

SPONSOR: CIBA-GEIGY Corporation

EXECUTIVE SUMMARY:

In a dermal penetration study (MRID 41833102), ^{14}C-CGA 24705 (% a.i. unknown) suspended in deionized water was applied to a 10 cm^2 area of the backs of 4 male Crl:CD®BR rats/group at doses of 0.01, 0.1 or 1.0 mg/cm^2. Each dose group was exposed for either 2, 4, 10 or 24 hours and then the area was washed and the animals sacrificed. Another 4 animals/dose group were treated for either 10 or 24 hours, the skin was washed and they were placed in a metabolism cage for collection of urine and feces. Sacrifice was 72 hours later. The amount of radioactivity

in the blood, urine, feces, carcass, skin and cage wash was determined for all animals.

CGA 24705 was rapidly absorbed with significant bioaccumulation. The total percentage of the applied dose which was found in the blood, urine, feces, carcass and cage wash (or absorbed) after 10 hours was 32.93, 20.26 and 6.98 at 0.01, 0.1 and 1.0 mg/cm^2, respectively. The percentage remaining on the skin was 24.66, 20.89 and 12.69 at the respective doses. The total percentage of the applied dose in the blood, urine, feces, carcass and cage wash (or absorbed) after 24 hours was 62.84, 26.85 and 16.15 at 0.01, 0.1 and 1.0 mg/cm^2, respectively. The percentage remaining on the skin was 11.09, 19.14 and 15.49 at the respective doses.

For rats with skin washings at 10 hours and sacrifice 72 hours after washing, the total percentage of the applied dose found in the blood, urine, feces, carcass and cage wash was 50, 38.61 and 15.46 at 0.01, 0.1 and 1.0 mg/cm^2, respectively. The percentage remaining on the skin was 5.30, 3.48 and 3.54 at the respective doses. For rats with skin washings at 24 hours and sacrifice 72 hours after washing, the percentage of the applied dose found in the blood, urine, feces, carcass and cage wash was 67.32, 43.46 and 30.49 at 0.01, 0.1 and 1.0 mg/cm^2, respectively. The percentage remaining on the skin was 3.39, 1.36 and 1.42 at the respective doses.

The study is classified as **Acceptable/guideline** and **satisfies** the guideline requirements for a dermal penetration study in rats (85-3; OPPTS 870.7600).

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Chemical:	**Metolachlor; (S)-2-Chloro-N-(2-ethyl-6-methylphenyl)-**
PC Code:	**108801; 108800**
HED File Code	***13000 Tox Reviews***
Memo Date:	**12/12/2001**
File ID:	**TX050330**
Accession Number:	**412-02-0281**

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