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

DEC - 1 1988

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Ignite®: Evaluation of the Toxicology Studies on Ignite® or Hoe 039866

Caswell No.: 580I
EPA Record No.: 203178/203179
Project No.: 8-0146
EPA ID No.: 8340-EO/ 8340-EI

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Registration Division (TS-767c)

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THROUGH: James N. Rowe, Ph. D. James N. Rowe 11/29/88
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In support of the registration of Ignite® as a herbicide for terrestrial food and non-food crop uses (see attached label), the registrant, Hoechst Celanese Corp., has submitted more than 42 studies. Some of these studies are not related to toxicology; the toxicologically relevant studies are evaluated. The data evaluation report of each study is attached, and the conclusions of the evaluated studies are presented below.

Based upon the EPA guidelines and the uses, the required toxicology studies for both terrestrial food and non-food uses are indicated in Tables I and II, respectively. In addition, the studies which have or have not satisfied the requirements are also shown in those two tables.

It should be noted that the mouse oncogenicity study and the combined chronic and oncogenicity rat study had been well conducted, but a NOEL for systemic toxicity could not be established in either study. Without a NOEL the rat study is not useful for regulatory purposes. The mouse study is considered as acceptable for assessing the oncogenic potential of the test agent. The rat teratology studies are available. When the data of these studies are combined, they are sufficient in characterizing the effects of Hoe 039866.

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Table I

Ignite®: The toxicology studies which are required for granting the registration of Ignite for food use and the studies which have satisfied the requirements.

Food Use		
Technical Product	Required	Satisfied
Acute oral LD_{50}	Yes	Yes
Acute dermal LD_{50}	Yes	Yes
Acute inhalation LC_{50}	Yes	Yes
Primary eye irritation	Yes	Yes
Primary dermal irritation	Yes	Yes
Dermal sensitization	Yes	Yes
90-day feeding - rodent	Yes	Yes
90-day feeding - non-rodent	Yes	Yes
21-day dermal	Yes	No
Chronic feeding		
rodent	Yes	No*
non-rodent	Yes	Yes
Oncogenicity		
rat (preferred)	Yes	No*
Mouse (preferred)	Yes	Yes†
Teratology		
rat	Yes	Yes
rabbit	Yes	No
Reproduction	Yes	Yes
Gene mutation	Yes	Yes
Structural aberration	Yes	No
other genotoxic effects	Yes	Yes
Metabolism	Yes	Yes

* The combined chronic feeding and oncogenicity study with rats has been well conducted, and the study was classified as minimum. However, a NOEL could not be established from the reported data. Also, the MTD was not approached for the male rats.

† The mouse oncogenicity study has been well conducted, and the study was classified as minimum. Although a NOEL for systemic toxicity could not be established from the reported data, the mouse study is considered to be acceptable for the purpose of assessing the oncogenic potential in mice.

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Table II

Ignite®: The toxicology studies which are required for granting the registration of Ignite for non-food use and the studies which have satisfied the requirements

Non-Food Use

Technical Product	Required	Satisfied
Acute oral LD_{50}	Yes	Yes
Acute dermal LD_{50}	Yes	Yes
Acute inhalation LC_{50}	Yes	Yes
Primary eye irritation	Yes	Yes
Primary dermal irritation	Yes	Yes
Dermal sensitization	Yes	Yes
90-day feeding - rodent (recommended)	Yes	Yes
21-day dermal (recommended)	Yes	No*
Teratology - rat (recommended)	Yes	Yes
Gene mutation	Yes	Yes
Structural aberration	Yes	No
Other genotoxic effects	Yes	Yes

* The use of Ignite® on turf grass will result in human exposure via skin contact.

CONCLUSION OF INDIVIDUAL STUDIES:

Subchronic Toxicity Studies

1). Subchronic dermal toxicity study in Wistar rats (Hoechst Study No. 83.0159; Report No. A31477; Project No. 84.0563)

Groups of Wistar rats (6/sex/dose) were repeatedly applied Hoe 039866 at doses of 0, 100, 300, and 1000 mg/kg/day on the shaved skin for 21 days. A recovery study applying low and high doses and 5 rats/sex was conducted for 14 days following 21 day dosing. The results indicated that 2/6 males of 300 mg/kg group and 4/11 males and 2/11 females in 1000 mg/kg groups showed aggressive behavior, piloerection, and a high startle response. Based on these observations, the LOEL is 300 mg/kg; NOEL, 100 mg/kg. The study is classified as Minimum.

2). 13-week feeding study in mice (Hoechst Report No. A31477; Project No. 018516)

When groups of NMRI mice (10/sex/dose) were fed diets containing Hoe 039866 at nominal concentrations of 0, 80, 320, and 1280 mg/kg for 13 weeks, significant increases in aminotransferase and in alkaline phosphatase were seen in high dose males. Increases in absolute and relative liver weights were also found in mid and high dose males. Based upon these findings LEL was established as 320 ppm; NOEL, 80 ppm. The study is classified as Minimum.

3). 28-Day feeding study in rats (Hoechst Report No. A34553; Project No. 772)

Groups of Wistar rats (40/sex) were administered Hoe 039866 at dietary concentrations of 0, 40, 200, 1000, and 5000 mg/kg diet for 28 days. The actual compound intakes were 0, 3.7, 18.7, 93.5, and 443 mg/kg body weight. Increased incidence of spontaneous activity, reactive behavior, and absence of pupillary reflex were seen in 5000 mg/kg males. Decreased body weight and food consumption were found in 5000 mg/kg males and in both 5000 mg/kg males and females, respectively. Decreased brain dopamine level was found in highest dose females. Decreased glutamine synthetase activity levels were seen in the liver of males in 200 mg/kg or more, in the kidneys of males in 200 mg/kg or more, and in the brain of 5000 mg/kg males. Based upon these findings, the LEL was 200 mg/kg diet (18.7 mg/kg bw/day; NOEL, 40 mg/kg diet (3.7 mg/kg bw/day).

The study design did not meet the requirements for a subchronic toxicity study. The study is classified as supplementary.

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4). 28-Day oral dose-range finding study in rats (Hoechst Report No. A29425; Project No. 018949)

Groups of Wistar rats (5/sex/dose) were fed Hoe 039866 at dietary concentrations of 0, 50, 500, 2500, and 5000 ppm for 28 days. At week 1 of treatment, food intake was decreased in 2500 and 5000 ppm males and females. An 11-20% increase of brain cholinesterase activity was seen in all treated females. Increases in urine specific activity of 50, 500, and 5000 ppm females and in absolute and relative kidney weights of all treated females were found. Since this study is a dose-range finding study, it is classified as supplementary.

5). 28-Day oral toxicity study in dogs (Hoechst Report No. A34048; RCC Project No. 048734)

This study consisted of two parts; one part dealt with metabolism, and the other part explored subchronic toxicity in the dogs.

Groups of beagle dogs (6/sex/dose) were orally administered (by capsule) Hoe 039866 in gelatin capsules at doses of 1 and 8 mg/kg/day. The animals received unlabelled Hoe 039866 technical on days 1-18 and then ^{14}C-Hoe 039866 on days 19 -28.

Metabolism: Most of the test material (>80%) was eliminated unchanged via feces; approximately 14-17% of the applied radioactivity was excreted in the urine. Of the radioactivity in the urine a metabolite, Hoe 061517, accounted for approximately 24.7%. The metabolism portion of the study is considered as acceptable.

Subchronic toxicity study: Slight to moderate inhibition of cerebral and hepatic glutamine synthetase activity was observed in 8 mg/kg/day dogs. This part of the study is considered as supplementary because there were many deficiencies which included no histopathology data.

5). Subchronic inhalation toxicity study in rats (Study No. 83. 0160; Report Issued: March 26, 1985)

Groups of Wistar rats (15/sex/dose) were exposed (nose only) to 0, 8, 20, or 46 mg/m^3 of Hoe 039866 for 28 days over a period of 40 days. The results indicated that increased coagulation time in females which received 20 or 46 mg/m^3. The NOEL was established at 8 mg/m^3. The study is considered as supplementary due to insufficient exposures to the test agent.

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Neurotoxicity Studies

1). Acute neurotoxicity study in white Leghorn hens (Hoechst Report No. A21969; Project No.: 275/79)

This study has two parts. One part explored the acute oral toxicity of Hoe 039866 in hens. In this portion of the study, groups of hens (2/dose) were orally administered (by gavage) Hoe 039866 liquid concentrate 40 at doses ranging from 1,600 to 10,000 mg/kg. Each hen received a single dose and was observed for 14 days. No acute toxicity was observed.

In another part of the study, 20 hens were divided into 4 groups with 5 hens in each group. Group 1 received a single oral dose of 10,000 mg/kg of Hoe 039866 liquid concentrate. Group 2 received the test substance plus antidote (atropine and toxogonin). Group 3 was the positive control which received triorthocresyl phosphate (500 mg/kg). Group 4 was negative control. The results showed show no abnormal behavior or histological changes in the brain, spinal cord, or sciatic nerve.

This study has many deficiencies, and it is classified as supplementary.

2). Observational assessment of neurotoxicity and determinaton of glutamine synthetase activity, NH_4^+ and glutamate levels in brain, liver, and kidneys of female Wistar rats (Hoechst Report No. A34242; Project No. 86.1003)

Groups of female Wistar rats (15/group or 30/group for high dose) were orally administered Hoe 039866 at doses of 200, 800, and 1600 mg/kg. Clinical and functional observations were carried out daily. Measurements of glutamine synthetase, NH_4^+, and glutamate levels were carried out. The results are the following:

a). Clinical signs. Increased spontaneous activity was seen in all treated animals. In 1600 mg/kg animals, increased incidences of spontaneous activity, piloerection, and tonoclonic convulsion were reported.

b). Functional observation for neurotoxicity signs: Decreased reaction was seen in 200 and 800 mg/kg animals. In 1600 mg/kg animals marked CNS excitability as indicated by convulsion and spasms followed by exhaustion was seen.

c). Decreased glutamine synthetase activity was seen in brain kidneys and liver essentially in all treated animals.

d). Changes in glutamate and NH_4^+ levels were variable in different organs examined.

This study has many deficiencies which include the use of only

female animals and difficulty in determining which animal showed which response. The study is classified as Supplementary.

Chronic Toxicity Studies

1). Chronic toxicity feeding study in dogs (Hoechst study No. A29827; Project No. 019203)

Groups of beagle dogs (main study, 8/sex; interim sacrifice, 4-3/sex) were fed Hoe 039866 in the diet at doses of 0, 2.0, 5.0, and 8.5 mg/kg/day. Two dogs in the 8.5 mg/kg group died during test, and the histopathology study showed that these animals had multiple myocardial necrosis and necrotizing aspiration pneumonia. At 6 months, electrocardiogram results revealed a dose related decrease in heart rates in treated males and females. Based on mortality, the LOEL is 8.5 mg/kg/day; NOEL, 5.0 mg/kg/day. This study is classified as Minimum.

2). Mouse oncogenicity study (Hoechst Report No. A33219; Project No. 018527)

Groups of NMRI mice (50/sex/dose) were fed Hoe 039866 at dietary concentrations of 20, 80, 160, or 320 ppm (160 ppm for males and 320 ppm for females) for 104 weeks. Interim sacrifice (10 mice/sex/dose) was carried out at 52 weeks.

Tumor incidence was comparable between treated and control animals. A dose-related increase in the mortality rate was seen in high dose males. In addition, significant decrease in body weight was found in high dose males of the interim sacrifice animals. Decreases in absolute liver weight and liver/body weight were found in all treated females. An increase in the incidences of cystic follicles and of chronic nephropathy in all treated males relative to those of the controls were noted. Based upon these findings, a NOEL could not be established. The study is classified as Minimum, but with no NOEL it is not useful for regulatory purposes.

3). Teratology study in rats (post-natal) (Hoechst Study No. 2R0486; Report No. A33812)

Twenty pregnant female Wistar rats were administered (by gavage) Hoe 039866 at doses of 0.5, 2.24, and 10 mg/kg from gestation days 7 to 16. The dams were allowed to deliver normally, and after birth the offspring were observed for 21 to 23 days. No compound related changes were seen in both maternal animals or offspring. The NOEL for maternal and developmental toxicity (post-natal) was 10 mg/kg/day (HDT). When the data of this study are considered with those of two previous studies (EPA Accession Nos.: 072965 & 073916), this study is classified as Minimum.

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4). Teratology study in rabbits (Study No. G2K0402; Project No. 84.0177)

Groups of pregnant Himalayan rabbits (15/sex/dose) were administered Hoe 039866 by gavage at doses of 2.0, 6.3, and 20 mg/kg from gestation days 7 to 19. Fetuses were delivered on day 29 by caesarean section. Increased incidences of premature delivery, abortion, or early resorption and decreased body weight and food consumption in 20 mg/kg dams were noted. In 20 mg/kg group, an increase in the number of dead fetus/litter was also seen. Increased incidence of weak or absent ossification of some skeletal bones in fetuses of 6.3 and 20 mg/kg groups was found.

The report has several deficiencies which include vague diagnoses of the skeletal examination. These deficiencies do not allow accurate assessment of the developmental toxicity of Hoe 039866 on rabbits, and under the present conditions a NOEL and LOEL are not established. This study is classified as Supplementary.

4). Two-generation reproduction study in rats (Hoechst Study No. A35589 and 33217)

In the F_0 generation, groups of Wistar rats (30/sex/dose) were fed Hoe 039866 at dietary concentrations of 0, 40, 120, and 360 ppm for 80 days prior to mating and throughout the gestation and lactation period. At the weaning of the offspring of F_{1B} generation, pups were selected to be the parents of F_{2A} and F_{2B} generations. The test compound containing diets were presented to all animals of all generations.

At 120 and 360 ppm, sigificant increases in kidney weights were seen in F_0 males and F_1 males and females, and based upon these findings the NOEL and LOEL for parental toxicity are established as 40 and 120 ppm, respectively.

At 360 ppm, significant reduction of the number of pups was observed in all generations. The LOEL for reproductive toxicity is 360 ppm, and NOEL is 120 ppm. This study is classified as Minimum.

5). Combined chronic toxicity and oncogenicity study in rats (Hoechst Report No. A33811; Project No. 018505)

Eighty Wistar rats/sex/dose were administered Hoe 039866 at dietary concentrations of 0, 40, 140, and 500 ppm. Ten rats/sex/dose were sacrificed at 52 weeks; 20 rats/sex/dose, at 104 weeks; 50 rats/sex/dose, at 130 weeks.

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Increased kidney glutamine synthetase activity and absolute and relative kidney weights in all treated females and in mid and high dose males were found.

Relative to the controls, a marginal increase in the incidence of pheochromocytoma was seen in male rats which were sacrificed at 130 weeks. This increase was within the range of the historical controls. In addition, pheochromocytoma was commonly seen in male rats. Therefore, an oncogenic effect of Hoe 039866 was not clearly demonstrated in this study.

Based upon the increases in glutamine synthetase activity of the kidney and in kidney weights in treated females of all dose levels, a NOEL could not be established. However, the study was well designed and conducted. Based upon the guidelines of the Agency, this study is classified as Core Minimum without a NOEL.

METABOLISM STUDIES

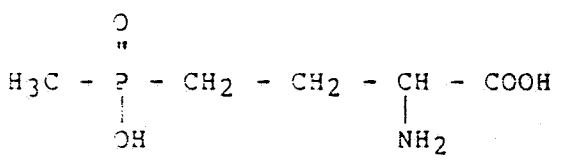
In the present submission there are 10 metabolism studies some of which are classified as supplementary. When all the data from these studies are analyzed together, the following metabolic characteristics of Hoe 039866 can be dervied:

a). With dermal application, the chemical is absorbed through the skin, much of the radioactivity is eliminated via urine, and the radioactivity is not accumulated in any organs examined.

b). With single oral dose administration, the majority of the radioactivity is excreted within the first 24 hrs, and much of it is eliminated in the feces. The excretion pattern is a two-phase process. The radioactivity level in liver, kidneys, brain, and gonads is minimal.

c). With repeated oral dosing, similar results are obtained as those with single oral administration.

d). Metabolite analyses show that in urine two metabolites are present in small quantities. One metabolite is Hoe 061517, and the other is Hoe 086486; the structures of both metabolites are presented below. In feces, essentially all the radioactivity is unchanged Hoe 039866.



Hoe 039866

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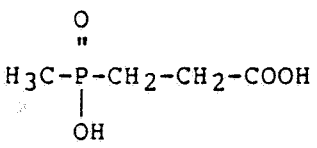
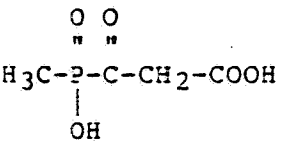


Hoe 061517



Hoe 086486

The available metabolism data on Hoe 039866 are sufficient in describing the metabolic fate of this compound even though some studies are classified as supplementary. Additional metabolism studies are not required at this time. The summary of each study is presented below.

1). Pharmacokinetic study with dermal application on rats (Hoechst Report No. A34502; Project No. 056597)

Twenty-eight male Wistar rats/dose were dermally applied radioactive Hoe 039866 at doses of 0.1, 1.0, and 10 mg/rat on 6 cm^2 of shaved skin. The 28 rats/dose were further divided into different subgroups which were exposed to the test agent for various times (o.5, 1, 2, 4, 10, 24, and 168 hrs). At the end of the exposure period the test agent was removed from the application site with soap and water wash except for 24 and 168 hr groups where the wash was after 10 hrs of exposure.

At low dose, 42.5 to 50.8% of the applied radioactivity was absorbed whereas at high dose 26% was absorbed.

After removal and washing of the treated skin, A substantial amount of the applied radioactivity still remained, and it was gradually absorbed and eliminated.

Accumulation of radioactivity was not found in any organ.

Radioactivity was found in both feces and urine, but majority of Hoe 039866 was eliminated via urine.

The study has been well conducted and reported; it is classified as Acceptable.

2). Studies of kinetics and residue determinatons in rats following an oral dose of 30 mg/kg (Hoechst Report No. A33239; Project No. 01-L42-0467-85)

Oral administration of a nominal dose (30 mg/kg) of ^{14}C-Hoe 039866 to 5 Wistar rats/sex resulted in rapid elimination during the first 24 hrs for both males and females. The major

route of excretion was via feces. Within 7 days of post dosing, greater than 94% of the dose was eliminated. Kinetics analysis revealed that the process of eliminaton was a two-phase process. The tissue radioactivity level for kidney, liver, and gonads was just above the background level.

Although this study did not identify any metabolite, the report presented valid kinetics data. It is clssified as Acceptable.

3). Studies of kinetics and residue determinations in rats after repeated oral dosing (2 mg/kg) (Hoechst Report No. A33975; Project No. 01-L42-0465-85)

Six Wistar rats/sex were orally administered (by gavage) unlabelled Hoe 039866 for 14 days and ^{14}C-Hoe 039866 at the 15th day at a dose of 2 mg/kg. Majority of the radioactivity was excreted within 24 hrs after the last dose. The major route of excretion was via feces. There was also a two-phase elimination process. More radioactivity was found in the tissues of animals dosed repeatedly than that of animals receiving a single dose.

Although this study did not present any findings on metabolites, it reported valid and useful information on the kinetics of the test agent in rats. It is classified as Acceptable.

4). Pharmacokinectics study in dogs (Hoechst Report No. A34282; Project No. 050185)

Two Beagle dogs/sex were orally administered (by capsule) ^{14}C-Hoe 039866 as a single dose (8 mg/kg). The results indicated that a small quantity of the test substance was absorbed, and the major route of excretion was via feces. Much of the excreted radioactivity consisted of the unchanged test article. Only a single metabolite, Hoe 061517, was found in the urine. Small amounts of radioactivity were found in the blood, plasma, heart, liver, kidneys, and various areas of the brain. This study has many deficiencies and is considered as a supplementary study.

5). Absorption, excretion, and metabolism of Hoe 039866 by dogs after repeated dosing (Hoechst Report No. A33914; Project No. 048734)

Six Beagle dogs/sex/dose were orally administered (by capsule) unlabelled Hoe 039866 for the first 18 days and ^{14}C-

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Hoe 039866 for another 10 days. The doses were 1 and 8 mg/kg. A small proportion of the radioactivity was absorbed. In 8 mg/kg dogs, radioactivity was found to enter the brain, and it could still be detected 4 days after the last dosing. Radioactivity was also found in kidneys and livers. The major route of excretion was via feces, and the main excreted compound was the unchanged Hoe 039866 in either feces or urine. A metabolite, Hoe 061517, was also detected in the urine. This study has many deficiencies and is classified as Supplementary.

6. Metabolism and residue determinations in female rats after repeated oral dosing (10 and 100 mg/kg)(Hoechst Report No. A33263; Project No.: CM007/85)

Groups of 15 female Wistar rats were administered labelled and unlabelled Hoe 039866 at different dosing intervals at doses of either 10 or 100 mg/kg. A total of 10 administrations were given. The results indicated that the major route of elimination was via feces. Unchanged test agent accounted for the majority of the excreted radioactivity in urine, feces, kidneys, liver, spleen, and brain. Metabolite, Hoe 061517, was found in urine, feces, and certain organs. Another metabolite, Hoe 086486 was detected in some urine and feces samples of 100 mg/kg animals. Repeated oral dosing of the test agent did not influence the pattern of excretion and residue accumulation in different organs.

The study is classified as Supplementary because the report presents only summary data which can not be verified.

7. Metabolism and residue determinations in rats after single oral administration (800 mg/kg) (Hoechst Report No. A32953; Project No. CM008/85)

Three Wistar rats/sex were administered (by gavage) ^{14}C-Hoe 039866 at a dose of 800 mg/kg. The results indicated that in urine, unmetabolized Hoe 039866 and two other metabolites, Hoe 061517 and Hoe 086486, were found. In feces, only unchanged Hoe 039866 was found.

This study is considered as supplementary because the data as presented are like summary data which can not be verified.

8. Metabolism in male and female rats after a single oral administration (2 mg/kg)(Hoechst Report No. A33895; Project No. CM011/86)

When 10 rats/sex were gavaged with ^{14}C-Hoe 039866 at a dose

of 2 mg/kg, greater than 80% of the applied radioactivity was excreted in the feces within the first 24 hrs. Unchanged Hoe 039866 practically accounted for all the excreted radioactivity. The pattern of excretion was similar in males and females.

This study has many deficiencies which include (1) the feces, urine, or tissue samples from all animals of each sex were pooled and examined as a single sample instead of examining the samples on an individual animal basis, (2) the results appeared to be derived from single determinations, and as such the data could only be considered as summary data which could not be verified, (3) the radioactivity in cage wash was never determined, and (4) the animal sacrifice date was never mentioned in the report. Therefore the study is classified as supplementary.

9). Metabolism in male and female rats after repeated oral administration of 2 mg/kg for 15 days (Hoechst Report No. A33893; Project No. CM082/85)

This study was conducted by the same group of reseachers as those of the study with Hoechst Report No. A33895 and with similar experimental design and deficiencies. It is classified as supplementary.

10). Metabolism in male and female rats after a single oral administration (30 mg/kg)(Hoechst Report No. A33894; Project No. CM083/85)

This study was conducted by the same group of reseachers as those of the study with Hoechst Report No. A33895 and with similar experimental design and deficiencies. It is classified as supplementary.

MUTAGENICITY STUDIES

In this submission 5 new mutagenicity studies are included. These studies are evaluated by Dynamac Corp. and approved by Toxicology Branch. The data evaluation report of each study is attached. In addition, an overview of all the submitted mutagenicity studies on Hoe 039866 has been prepared by Kerry Dearfield (Attachment 1).

Summary:

The available mutagenicity data on Hoe 039866 do not indicate a mutagenicity concern, but they have not entirely satisfied the regulatory requirements for the mutagenicity testing. "Acceptable

results have satisfied the requirements for examining gene mutations (Salmonella, E. coli and mouse lymphoma assays) and other genotoxic effects (UDS). However, there are no acceptable studies to satisfy the requirements for structural aberrations" which is a data gap.

SPECIAL STUDIES

1). Investigating the effects of Hoe 039866 and Hoe 035956 on various enzymes in vitro (Hoechst Report No. A34404; Project No. KO 3/1986)

The possible inhibitory effects of Hoe 039866 and Hoe 035956 on glutamate oxalacetate transaminase (GOT), glutamate pyruvate transaminase (GPT), glutamate dehydrogenase (GDH), and gama-glutamyl transpeptidase (GGTP) were examined in vitro. The results showed that 10 mmol/L Hoe 039866 did not affect the functions of these enzymes examined. This study is classifies as supplementary.

2). Testing the therapeutic effects of atropine sulphate and 2-PAM on Hoe 039866 treated rats (Hoechst Report No. A34188; Project No. 86.0973)

Atropine sulphate and 2-PAM-methiodide did not produce any therapeutic effect on rats which were treated with a lethal dose of Hoe 039866 (males, 3200 mg/kg; females, 2200 mg/kg). This study provides useful information. It is a special study and is classified as supplementary.

3). In vitro receptor binding assays (Hoechst Report No. A34303; Project No. 86.1243)

The results of the receptor binding assays indicated that 1 uM Hoe 039866 did not bind significantly with any of the receptors examined (GABA, NA-beta, 5-HT_1, 5-HT_2, Ca^{2+} channel, and benzodiazepine receptors). The study is considered as supplementary.

4). Determination of glutamine synthetase (GS) activity, NH_4^+, glutamine, and glutamate levels in brain, liver, kidney, and heart of Hoe 039866 treated rats and mice (Hoechst Report No. A34503; Project No. 85.0066)

Groups of 5 female rats or mice were orally administered Hoe 039866 at doses of 50 and 200 mg/kg for mice and 200 and 800 mg/kg for rats. The animals were sacrificed 4 hrs after dosing. No changes in brain GS activity were seen in both treated rats and mice. Significant decrease in kidney GS activity was seen in all treated rats and mice. Reduced liver GS activity was found in 800 mg/kg rats. Heart GS activity was elevated in mice.

No significant changes in NH_4^+, glutamine, and glutamate levels were found in rats or mice. This study is classified as supplementary.

5). Catecholaminee and glutamine synthetase determinaitons on Hoe 039866 and Hoe 061517 treated rats (Hoechst Report No. A34318; Project No. 86.1190)

In Wistar male rats, intracerebral (i.c.v.) injection of 20 ug Hoe 039866 caused severe convulsions which were reported to be ameliorated by diazepam (10 mg/kg, i.p.). A dose of 10 ug Hoe 039866 caused spasm of the forelimbs.

With i.c.v. injection of 20 ug Hoe 061517, a major metabolite of Hoe 039866, spasm of the forelimbs was also seen.

Changes in brain catecholamines were observed in 20 ug rats (i.c.v.), but the biological significance of these changes were not clear.

With i.c.v. injection, Hoe 039866 at doses of 10 and 20 ug significantly inhibited brain glutamine synthetase activity, and the inhibition was dose-dependent.

Intravenous injection of Hoe 039866 at doses of 10 and 20 mg/kg did not produce significant changes in the levels of brain catecholamines or of glutamine synthetase activity. The study is classified as supplementary.

6). Effects of Hoe 039866 on the function of rat liver mitochondria (Hoechst Report No. A34359)

The results indicated that Hoe 039866 either did not affect the function of rat liver mitochondria or it could not be taken up by this organelle. This study is classified as supplementary.

7). Inhibition of liver glutamine synthetase by phosphonic analogues of glutamic acid (Hoechst Report No. A32191)

The relevant results indicate that analogues of glutamic acid and glutamine in which alpha- or gamma-COOH goups were substituted by PO_3H_2 or $P(O)(CH_3)OH$ groups competitively inhibited rat liver glutamine synthetase activity. The report is a publisned article, and the data can not be verified. The study is considered as inacceptable.

8). Inhibition of glutamate decarboxylase by phosphoni analogues of glutamic acid (Hoechst Report No. A34389)

The relevant results indicate that 2-amino-4-(methylphosphino)butyric acid, which is structurally similar to Hoe 039866, inhibited mammalian brain glutamate decarboxylase. The report is a published article, and it presents mainly summary data which can not be verified. The study is unacceptable.

9). General pharmacology study on Hoe 039866 (Hoechst Report No. A34071)

This report contains several studies some of which can be considered as parts of a chronic toxicity or a neurotoxicity study while others are irrelevant to toxicology. A detailed data evaluation is not prepared for this study.

FORMULATION

1). Contact hypersensitivity study in guinea pigs with liquid formulation of Hoe 039866 (Hoechst Report No. A32847; Project No. 053010)

The test article did not cause skin hypersensitivity in guinea pigs, and the study is classified as Minimum.

ATTACHMENT 006936

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

MAY 5 1988

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Overview of Submitted Mutagenicity Studies on Ignite

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Chief
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Chemical: Ignite CAS# 77182-82-2 Caswell #580I

This reviewer has been requested to examine and summarize mutagenicity studies concerning Ignite that have been submitted to OPP. The following is a listing of these studies with their result and classification for acceptance:

Acceptable studies:

Salmonella assay: negative, Document #004403
E. coli reverse mutation assay: negative, Document #004403
Mouse lymphoma assay: negative, new DER
UDS/primary rat hepatocytes: negative, new DER

Unacceptable studies:

Salmonella assay: another assay from one above, negative, Document #004403
B. subtilis rec assay: negative, Document #004403, however, results from non-activated portion are adequate
Mouse micronucleus: negative, Documents #004403 & #004928
S. pombe forward mutation assay: negative, new DER

S. cerevisiae mitotic gene conversion: negative, new DER
Chromosome aberrations in cultured human lymphocytes: negative, new DER

The following will discuss these studies and then present an overall conclusion.

A. Salmonella assays

Two Salmonella assays were considered in Document #004403. In the acceptable study, Salmonella strains TA98, TA100, TA1535, TA1537 and TA1538 were exposed to test substance ± activation at concentrations up to 1000 ug/plate. There was virtual inhibition of growth noted in all strains at 1000 ug/plate. The results were negative for inducing an increased mutation frequency.

The second Salmonella assay was considered unacceptable. The same five strains were used and exposed to 0.5 ul/plate and negative results also obtained. While toxicity may be apparent at the highest concentration tested as the number of revertants dropped, the original reviewer considered this assay to not demonstrate toxicity (to upgrade this assay, the registrant should detail this aspect more fully). Other reasons for an unacceptable decision were actual concentrations of test material were not reported (only volumes provided) and the technical grade of the compound was not used.

B. E. coli reverse mutation assay (Document #004403)

An acceptable E. coli reverse mutation assay was performed with strain B/r WP2 hcr^- try^- at concentrations up to 1000 ug/plate. Virtual inhibition of growth was noted at the top concentration. The results were negative for inducing an increased mutation frequency.

C. Mouse lymphoma assay (new DER)

Test substance at concentrations up to 5000 ug/ml ± activation did not increase the mutation frequency after a 4 hour exposure. Top toxicities at 5000 ug/ml ranged from 28.3% to 73.6% relative growth (5000 ug/ml is a top recommended test concentration for this test).

D. Unscheduled DNA synthesis (UDS) in primary rat hepatocytes (new DER)

Test substance at concentrations up to 5240 ug/ml did not induce UDS after an 18-19 hour exposure. The top concentration produced 39.5% survival. The positive control was adequate.

E. B. subtilis rec assay (Document #004403)

Strains H17 (rec^+) and M45 (rec^-) were exposed to test

substance at concentrations up to 10,000 ug/plate under non-activated conditions only. There were no differences in growth between the two strains, indicating no detectable genotoxic effect. The original review states that this assay is acceptable, however, since activated conditions were not utilized, this assay should be considered unacceptable. However, the results without activation are adequate.

F. Mouse micronucleus assay (Documents #004403, #004928)

Five NMRI mice/sex/treatment group were exposed to 2 doses of 0, 8, 40 or 200 mg/kg of test substance by gavage 24 hours apart. They were sacrificed 6 hours after the second administration and bone marrow examined for micronuclei. In Document #004403, this assay was originally considered unacceptable as dosing did not appear justified (i.e. higher dosing may have been necessary). Document #004928 upgrades this assay to acceptable as a later submission provides the rationale for dose selection. Doses of 250 or 300 mg/kg/day produced clinical signs or were outright lethal to male and female mice. It was concluded that 200 mg/kg/day was a sufficient top dose.

However, this assay should remain as unacceptable. While the dosing may have been determined to be adequate, the protocol is not an acceptable one on a scientific basis. The sampling of bone marrow only six hours after the last dose does not allow adequate time for micronuclei to form from the last administration, and the sampling time does not allow for possible effects on cell cycle time to be taken into account. It is recommended that sampling of bone marrow should occur three times from at least 12 hours to up to 72 hours after the last dosing.

G. Schizosaccharomyces pombe forward mutation assay (new DER)

Concentrations up to 1000 ug/ml (based on a preliminary toxicity assay - no details given) were exposed for 4 hours to S. pombe. Survival was at 86% or greater in contrast to the recommended survival of at least 50% for a maximum dose. No mutagenic effect ± activation was observed with little concurrent toxicity. The original reviewer suggests that optimal conditions for expression were not provided; the stationary phase culture used was incubated at 32° C for 48 hours while recommended incubation is at 30° C for 36 hours to achieve optimum sensitivity.

H. Mitotic gene conversion in Saccharomyces cerevisiae D4 (new DER)

Concentrations of test substance of 1000, 2500, 5000 or 10,000 ug/ml ± activation did not increase conversion at the tryptophan or adenine loci. The cells were exposed for 4 hours and 32% survival was noted at the top concentration without activation and 68% survival with activation. However, the assay

was performed with stationary phase cultures, therefore the physiological state may have compromised the sensitivity of the assay. It is recommended that logarithmic phase cultures should be used to increase the sensitivity of the assay.

I. Chromosome aberration assay in cultured human lymphocytes (new DER)

Singly prepared cultures from one volunteer were incubated with 1, 10, 100 or 1000 ug/ml ± activation for 3 hours with test substance (after a 48 hour incubation from culture initiation in the presence of PHA). The cultures were washed and incubated for an additional 23 hours before colchicine was added for 3 more hours of incubation. A preliminary toxicity experiment showed that 55% of the control mitotic activity was noted at the top concentration without activation (activated conditions not tested). Over 90 metaphases/culture were scored and the positive controls appeared adequate. No increase in aberration frequency was observed.

This assay was considered unacceptable since only one culture per treatment group was established. It is recommended that two independent cultures be established per treatment group. Other problems with this assay include no toxicity established for cultures in the presence of activation (appears in this case the toxicity may be different and therefore different concentrations could have been tested). Also, the original reviewer suggests that the method of scoring slides may not be totally adequate. Aberrations were scored from projected film slides which may limit analysis to a single plane; the preferred method is to score slides directly from the microscope to examine all planes in the three dimensional preparation. Finally, slides were not apparently coded.

Overall Conclusions

Based on the weight-of-evidence from the submitted studies on Ignite, there does not appear to be a mutagenicity concern for Ignite at this time. However, the regulatory requirements have not been totally satisfied for the mutagenicity testing of Ignite. Acceptable results have satisfied the requirements for examining gene mutations (Salmonella, E. coli and mouse lymphoma assays) and other genotoxic effects (UDS). However, there are no acceptable studies to satisfy the requirement for structural aberrations. This is a data gap for regulatory purposes.

cc: M. van Gemert
Section Chief

RIN 5218-93 / Tox Review for Glufosinate Log #293

Page_____ is not included in this copy.

Pages 21 through 35 are not included.

The material not included contains the following type of information:

____ Identity of product inert ingredients.

____ Identity of product impurities.

____ Description of the product manufacturing process.

____ Description of quality control procedures.

____ Identity of the source of product ingredients.

____ Sales or other commercial/financial information.

__✓__ A draft product label.

____ The product confidential statement of formula.

____ Information about a pending registration action.

____ FIFRA registration data.

____ The document is a duplicate of page(s) _____.

____ The document is not responsive to the request.

The information not included is generally considered confidential by product registrants. If you have any questions, please contact the individual who prepared the response to your request.

Reviewed by: Byron T. Backus Byron T. Backus 9/12/88
Section 3, Tox. Branch (TS-769C)
Secondary reviewer: Marcia van Gemert
Section 3, Tox. Branch (TS-769C) Marcia van Gemert 9/16/88

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DATA EVALUATION REPORT I

STUDY TYPE: 28-day oral dosing - dog TOX. CHEM. NO: 580 I

ACCESSION NUMBER: 403456 MRID NO: not given

TEST MATERIAL: HOE 039866 Technical

SYNONYMS: Ignite™

STUDY NUMBER(S): RCC Project No. 048734; Report No. A34048

SPONSOR: Hoechst Celanese Corporation, Somerville, NJ

TESTING FACILITY: RCC (Research & Consulting Company AG)
Itingen, Switzerland

TITLE OF REPORT: 28-day oral toxicity (capsule) study in the dog with HOE 039866 Technical (Code: HOE 039866 OH ZC95 0001) and 14C-HOE 039866 with special reference to mode of action and target organ

AUTHOR(S): Sachsse, K., Frei, T., Luekemeier, H., Ellgehausen, H., Gembardt, C., and Terrier, C.

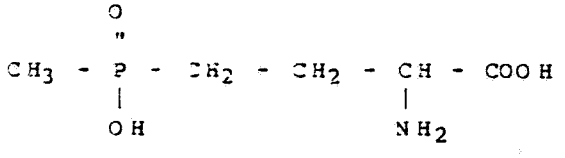
REPORT ISSUED: September 16, 1986

Classification: (as a subchronic dosing study): core supplementary
(as a metabolism study): acceptable

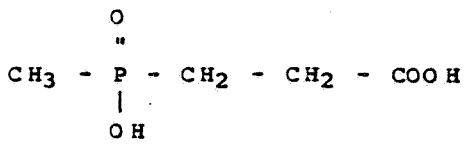
Special Review Criteria (40 CFR 154.7)

CONCLUSIONS:

1. The study demonstrates that when administered orally to dogs (at doses of 1 and 3 mg/kg/day) most of the test material is eliminated unchanged via the feces, with about 14 to 17% excreted via the urine. Overall >95% of the label was eliminated or excreted. Most of the material excreted in the urine was the parent compound:



However, a considerable proportion (up to 24.7% in the 8 mg/kg/day dogs) was the metabolite HOE 061517, with the following structure:



This was the only metabolite that was detected, or that was apparently present as a significant (>5%?) part of the total radioactivity.

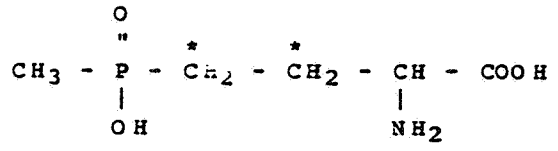
2. It is reported (p. 14) that the "slight to moderate inhibition of cerebral and hepatic glutamine synthetase" in 8 mg/kg/day dogs was "due to competitive interference related to the structural analogy of the test article with glutamate... This enzyme inhibition was partly associated with a decrease of glutamine levels in the tissues and increased alpha-keto-glutarate levels in these organs. In respect to the decreased levels of phosphoethanolamine in some brain areas, it was not not possible to assess the physiological relevance of this finding."

3. As a metabolism study, the study is classified acceptable, but it is noted that this study by itself would not fully meet Agency metabolism data requirements if the active was to be used on food crops. However, the cover page indicates that the study is being submitted in support of an application for registration as a herbicide for non-food crop uses.

4. As a subchronic feeding study, this report is classified as core supplementary data. A subchronic feeding study for the dog should usually be 90 days (rather than 28 days) and should preferably utilize at least 3 dose groups rather than the 2 which were present in this study. Also, there was a lack of any significant toxicologic effect at the high dose level (8 mg/kg/day), and no histopathology examination results were conducted. The the finding of dose-related and significant decreases in mean phosphoethanolamine levels for several brain tissues (although this is not a parameter that is usually measured, and the toxicological significance is obscure) indicates that a NOEL was not observed at the low dose level (1 mg/kg/day).

A. MATERIALS:

1. Test compound: HOE 039866 Technical, Description: a white hygroscopic powder; batch # HOE 039866 OH ZC95 0001 and HOE 039866 OT ZE98 0005 (^{14}C-labeled article); purity 95.3% and 98% respectively. The structure of the radiolabeled compound is given below:



The specific radioactivity of the labeled compound was 54.08 mCi/g.

2. Test animals: Species: dog; Strain: Purebred beagle; age: 4-7 months; weights: males 5.0-6.2 kg, females 3.7-7.3 kg at pretest; source: KFM Kleintierfarm Madoerin AG, CH 4414 Fuellinsdorf, Switzerland.

B. STUDY DESIGN:

1. Animal assignment

Beagle dogs were assigned to groups to give an even weight distribution and to eliminate bias due to sibling relationship (10 additional animals were allocated for this purpose), as indicated below:

Group	Dose in capsule (mg/kg/day)	Main Study 28 days male	female
1 Control	0	6	6
2 Low (LDT)	1	6	6
3 High (HDT)	8	6	6

One male and one female of each group were sacrificed on days 18, 19 and 28 and at days 1, 2 and 4 of the recovery period.

2. Dosage: The test article was weighed into gelatin capsules with adjustment for individual body weights. Dogs were dosed daily following the feeding period. The animals received unlabeled HOE 039866 technical on days 1-18 and ^{14}C-labeled HOE 039866 on days 19-28.

3. Animals received food [Kliba no. 335 dog maintenance diet (Kliba Futter, Klingentalmuehle AG, Switzerland)], and water ad libitum.

4. Statistics - See the appended sheet I (from p. 40) for the methods used to analyze the data.

5. A signed and dated quality assurance unit statement is given on p. 9, listing the dates of QAU inspections/audits and the subsequent dates for reports. A signed statement of compliance to good laboratory practice appears on p. 10 of the report.

C. METHODS AND RESULTS:

1. Observations:

Animals were inspected twice daily for signs of toxicity and mortality.

Results:

From p. 12: "No animal died during the study."

"Detailed observations and neurological examinations yielded no treatment-related changes. Only a slightly increased spontaneous motor activity of the animals of group 3 was noted, which might be indicative for a stimulation of central nervous function."

2. Body weight:

"The body weight of each animal was recorded once weekly using a Mettler PK 60 balance."

The mean body weights (in grams)/sex/group (calculated from data on p. 189-190; also presented on p. 58-61) are given below:

	Males			Females		
Group	I	II	III	I	II	III
Dose (mg/kg/day)	0	1	8	0	1	8
Pretest week 1	5483	5774.3	5726	4655	4197.7	4637
Pretest week 2	5498.3	5978	5889.7	4751.7	4404.3	4738
Day 7	5674	6082.5	5822	4832	4447.5	4722.5
Day 14	5811.3	6297	5984	5160	4667	4834.5
Day 21	6074.5	6336.5	6088	5371	4709	4866.5
Day 28	6257	6387.5	6248	5533	4791	4965

This reviewer has recalculated the mean body weights given above, using only values from those 4 dogs/sex/group not sacrificed between days 14 and 21:

Group	Males			Females		
Dose (mg/kg/day)	I 0	II 1	III 8	I 0	II 1	III 8
Pretest week 1	5629	5768.5	5646	4937.5	3968	4597
Pretest week 2	5695	5910	5915	5037.5	4160	4660
Day 7	5889.5	5983	5838.5	5242	4259	4643.5
Day 14	6027	6159	5984	5393	4477	4709
Day 21	6074.5	6336.5	6088	5371	4709	4866.5
Day 28	6257	6387.5	6248	5533	4791	4965

The following are the mean % weight gains:

Group	Males			Females		
Dose (mg/kg/day)	I 0	II 1	III 8	I 0	II 1	III 8
Means of all dogs:						
Pretest week 1 to day 28	14.1	10.6	9.1	18.9	14.1	7.1
Pretest week 2 to day 28	13.8	6.9	6.1	16.4	8.8	4.8
Means of only those dogs not sacrificed days 18-19:						
Pretest week 1 to day 28	11.2	10.7	10.7	12.1	20.7	8.0
Pretest week 2 to day 28	9.9	8.1	5.6	9.8	15.2	6.5

On p. 12 it is stated that "the body weight of the group 3 males was reduced during the first week of treatment and that of the corresponding females during the entire treatment phase." However, those group 3 males not sacrificed during the study had a comparatively large mean weight gain in the period from pretest week 1 to pretest week 2, but then there was an actual drop in mean weight from pretest week 2 to study week 1 for this group, with overall weight gains from pretest week 1 to day 28 similar for all 3 male groups. For group 3 females there was a reduced mean weight gain during the dosing period.

With respect to body weights and body weight gains, it is stated (p. 183) "There were no significant differences between the treated groups and the control groups."

3. Food consumption:

"Food consumption was recorded daily... The weekly mean of all individual values and the group mean of these values are reported."

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Results: From p. 41: "During the treatment period, the mean food consumption was similar in all male groups and slightly decreased in the females of groups 2 and 3 when compared to the control group."

Although mean food consumption was lower in the two dosed female groups, particularly at the start of the study, one female (#299) in the control group was larger (>7 kg) than any other female in the study (none over 6 kg). Food consumption is also expressed as g/animal/day (rather than in terms of g/kg/animal/day).

With respect to food consumption, it is stated (p. 183) "There were no significant differences between the treated groups and the control groups after adjustment for multiple testing."

4. Ophthalmological examination

All eyes were examined pretest, and again at 14 and 25 days.

Results: From p. 41: "Some abnormalities, such as superficial opaque spots on the cornea, granular particle on the anterior surface of the lens, redness of the conjunctivae and purulent discharge were noted in some dogs both at pretest and at 14 and 25 days of treatment. The findings were considered to be spontaneous in nature, as they are commonly observed in the Beagle stock used..."

5. Blood was collected before treatment and on days 11, 28 and (from the one non-sacrificed dog/sex/group left) at 4 days after the last dose, for hematology and clinical analysis. The CHECKED (X) parameters were examined:

a. Hematology

X		X	
X	Hematocrit (HCT)*	X	Leukocyte differential count
X	Hemoglobin (HGB)*	X	Mean corpuscular Hemoglobin (MCH)
X	Total Leukocyte count (WBC)*	X	Mean corpuscular HGB conc. (MCHC)
X	Erythrocyte count (RBC)*	X	Mean corpuscular volume (MCV)
X	Platelet count (PLT)*	X	Reticulocyte count
	Blood Clotting Measurements	X	Nucleated erythrocytes (NEN)
X	(Thromboplastin time)	X	Red cell morphology
	(Partial thromboplastin		Methemoglobin (METHGB)
X	time)		Heinz bodies

* Required for subchronic and chronic studies

"Tissue sections for the analysis of glutathione, glutamine synthetase, alpha-ketoglutarate, amino acids, and brain cholinesterase were taken at necropsy. The tissue sections were weighed, rinsed in ice-cold saline (0.9% NaCl) solution, blotted dry, and immediately frozen in liquid nitrogen, and stored at -20 degrees centigrade until analysis. The tissues examined were liver..., heart..., kidney..., four regions of the brain (cortex, midbrain (includes thalamus), cerebellum and brain stem), and spinal cord."

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Results:

From p. 42: There were..."no changes of toxicological significance at 11 and 28 days of treatment, nor at the end of the 4-day recovery period. The slightly decreased values noted in the erythrocyte count, hemoglobin concentration and hematocrit value for the female dogs of groups 2 and 3 at 28 days, and at the end of the 4-day recovery period, were considered as secondary effects and not treatment-related..."

"All other statistical differences in the results of the hematology parameters were considered to be incidental and of normal biological variation."

b. Clinical Chemistry

The CHECKED (X) parameters were examined. Where (tissue) is indicated, samples were taken from sacrificed animals:

X	Electrolytes:
X	Calcium*
X	Chloride*
	Magnesium*
X	Inorganic Phosphorus*
X	Potassium*
X	Sodium*
	Enzymes:
X	Alkaline phosphatase
X	Plasma Cholinesterase#
X	RBC Cholinesterase#
X	Creatinine phosphokinase**
X	Lactate dehydrogenase
X	Serum alanine aminotransferase (ALT; also SGPT)*
X	Serum aspartate aminotransferase (also SGOT)*
X	Gamma glutamyl transferase
X	Glutamate dehydrogenase
X	Glucose-6-phosphate dehydrogenase
X	Glutathion reductase
X	Glutamine synthetase
X	Alpha-ketoglutarate

X	Other:
X	Albumin*
	Albumin/globulin ratio
X	Blood creatinine*
X	Blood urea nitrogen*
X	Total Cholesterol*
	Globulins
X	Glucose*
X	Total Bilirubin*
X	Total Serum Protein*
	Triglycerides
	Serum protein electrophoresis
X	Triiodothyronine (T3)
X	Thyroxine (T4)
X	Blood gases (Acid-base status)
X	pH
X	partial pressure of CO_2
X	partial pressure of O_2
X	Base excess (extracellular)
X	Base excess (blood)
X	Actual bicarbonate
X	Standard bicarbonate
X	Buffer base
X	Glutathion (reduced)
X	Glutathion (oxidized)
X	Glutathion (total)
X	Free amino acids (in plasma)
X	Free amino acids (in tissue)

* Required for subchronic and chronic studies
\# Required for OP
° Not required for subchronic studies

Results:

For blood (p. 42): There were "...no changes of toxicological significance...at 11 and 28 days of treatment, nor at the end of the 4-day recovery period."

For tissues (p. 42-44): "...analysis of tissue extracts for levels of glutathione, glutamine synthetase activity, alpha-ketoglutarate activity and amino acids indicated the following treatment-related changes:"

An asterisk (*) indicates that the value is reported as statistically different ($p < 0.05$) from that of the control.

Decreased midbrain glutathione levels in group 3 females:

From p. 93:

	Reduced glutathione umol/g	Oxidized glutathione umol/g	Total glutathione umol/g
Female group 1	0.01	0.51	0.52
Female group 2	0.04	0.46	0.50
Female group 3	0.03	0.22*	0.26*

Inhibition of midbrain and cerebellum glutamine synthetase activity occurred in group 3 males and females, and for spinal cord in group 3 males:

Glutamine synthetase activity (umol gamma glutamyl-hydroxamate formed per ml reaction mixture/20 min/37° C).

	From p. 97 - Females			From p. 91 - Males		
	Midbrain	Cerebellum	Spinal Cord	Midbrain	Cerebellum	Spinal Cord
Gp 1	4.44	3.22	1.01	4.52	3.64	1.10
Gp 2	4.88	3.39	1.05	4.49	3.58	0.90
Gp 3	3.20*	2.16*	0.70	2.93*	1.72*	0.55*

It is reported (p. 43) that "A trend toward slight inhibition of cortex glutamine synthetase activity was noted for male dogs of group 3, and of liver glutamine synthetase activity for male and female dogs of group 3." None of these "trends" involved statistically significant differences between groups.

There was increased cerebellar alpha ketoglutarate activity (expressed below as umol alpha-ketoglutarate/g wet weight/13 min) in group 3 males and females:

	From p. 98 - Females Cerebellum	From p. 92 - Males Cerebellum
Gp 1	0.032	0.032
Gp 2	0.045	0.029
Gp 3	0.069*	0.048*

Dose-related and significant decreases in mean glutamine levels for several tissues were noted, particularly for females (values below in umol/g):

	From p. 95 - Females			From p. 89 - Males
	Heart	Cortex	Brain Stem	Brain Stem
Gp 1	15.23	5.14	2.13	2.35
Gp 2	12.40*	4.55	2.27	2.27
Gp 3	12.99*	3.76*	1.69	1.78*

Dose-related and significant decreases in mean taurine levels for several tissues were noted, particularly in females (values below in umol/g):

	From p. 97 - Females				From p. 91 - Males
	Heart	Cortex	Cere-bellum	Brain Stem	Brain Stem
Gp 1	11.08	2.15	1.23	1.25	2.13
Gp 2	7.72	0.98*	1.07	0.95	2.08
Gp 3	6.26*	0.48*	0.68*	0.70*	1.54*

Dose-related and significant decreases in mean phosphoethanolamine levels for several tissues, particularly the cerebellum, were noted (values below in umol/g):

	From p. 96 - Females			From p. 90 - Males
	Cortex	Cere-bellum	Brain Stem	Cere-bellum
Gp 1	2.51	0.53	0.81	1.26
Gp 2	1.25*	0.71	0.67	0.67*
Gp 3	0.77*	0.13*	0.40*	0.02*

Possible (but sporadic) effects also occurred involving levels of arginine, aspartic acid, glutamic acid and glycine different organs.

6. Urinalysis

Urine samples were collected from all dogs following 18-hr fasting on days 11 and 28, and after the 4-day recovery period. "Urine was collected into a specimen vial using a catheter and divided into two aliquots. One for the standard analysis, and one acidified to a pH of 1-2 and used in the analysis of catecholamines and 5-hydroxyindole acetic acid." For the standard analysis, the CHECKED (X) parameters were examined.

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

* Required for chronic studies

Also, quantitative analyses were performed (refer to p. 37-38) for the following:

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Creatinine
Catecholamines
Dopamine (Dihydroxyphenylethylamine) (DOPA)
Norepinephrine (Noradrenaline)
Epinephrine (Adrenaline)
Vanilmandelic acid (VMA)
Homovanillic acid (HVA)
5-Hydroxyindole acetic acid (5-HIAA)

Results:

From p. 45: "The assessment of urinalysis data...indicated no changes of toxicological significance at 11 and 28 days of treatment, nor at the end of the 4-day recovery period."

7. Sacrifice and Pathology -

"Samples of the following tissues and organs were collected from all animals at necropsy and fixed in neutral phosphate buffered 4% formalin; eyes were fixed in Heidenhain's Susa solution." On p. 38 it is stated that histopathological examination was not performed. (X indicates tissues were collected). The (XX) organs were weighed.

X	Digestive system
X	Tongue
X	.Salivary glands*
X	.Esophagus*
X	.Stomach*
X	.Duodenum*
X	.Jejunum*
X	.Ileum*
X	.Cecum*
X	.Colon*
X	.Rectum*
XX	.Liver*†
X	Gall bladder*#
X	.Pancreas*
	Respiratory
X	.Trachea*
X	.Lungs*

X	Cardiovasc./Hemat.
X	.Aorta*
XX	.Heart*
	.Bone marrow*
X	.Lymph nodes*
X	.Spleen*
X	.Thymus*
	Urogenital
XX	.Kidneys*†
X	.Urinary bladder*
X	.Testes*†
X	.Epididymides
X	.Prostate
	.Seminal vesicle
X	.Ovaries*†
X	.Uterus*
	.Cervix
X	.Vagina

X	Neurologic
XX	.Brain*†
X	.Sciatic nerve*#
X	.Spinal cord (cervical)*#
X	.Pituitary*
X	Eyes*#
X	Orbital gland
	Glandular
X	.Adrenals*
	.Lacrimal gland#
X	.Mammary gland*#
X	.Parathyroids*
X	.Thyroids*
	Other
X	.Bone*#
X	.Skeletal muscle*#
X	.Skin*#
X	.Rib with cartilage
X	.All gross lesions and masses*

* Required for subchronic and chronic studies
\# In subchronic studies, examined only if indicated by signs of toxicity or target organ involvement
† Organ weights required in subchronic and chronic studies

Results:

a. Organ weight

From p. 45: "The organ weights, organ to body weight as well as organ to brain weight ratios were comparable between all animals."

b. Gross pathology

From p. 45: "At necropsy, there were no treatment-related macroscopical changes observed."

c. Microscopic pathology

On p. 38 it is stated that histopathological examination was not performed.

8. Metabolism:

From day 19 to 28 dogs were treated with capsules containing radiolabeled (14C) HOE 039866. Some of the dogs were sacrificed 6 hours after the first treatment, while others were followed for 96 hours for urinary and fecal excretion of the radioactivity. Blood samples were also taken at different times. The following sacrifice schedule was used, with one male and one female/dose group being euthanized on each date:

Day	Hours after administration of 14C-HOE 039866	Number of dogs sacrificed on that date: Males (mg/kg) 0	1	8	Females (mg/kg) 0	1	8
18	Prior to dosing	1	1	1	1	1	1
19	6*	1	1	1	1	1	1
28	6**	1	1	1	1	1	1
29	24**	1	1	1	1	1	1
30	48**	1	1	1	1	1	1
32	96**	1	1	1	1	1	1

* After first administration of ^{14}C-HOE 039866
**After last administration of ^{14}C-HOE 039866

Livers, kidneys, hearts and several brain regions of male and female dogs sacrificed at various times were analyzed for radioactivity. Liver and kidney of high-dose males and females sacrificed 6 and 24 hours after the last administration of radioactively-labeled material were also analyzed.

Results:

Blood:

Measured amounts in the 1 mg/kg/day group were in the range from 0.023-0.089 mcg parent equivalents per gm. In the 8 mg/kg/day group the range was from 0.035 to 0.450. The highest values obtained tended to be on or about day 28. The concentration of radioactivity was higher in plasma than in the blood. In a male of the high dose group the half-life in the blood was 46.2 hours and in the plasma was 16.1 hours. In high-dose females the half-life in combined blood and plasma was less than 18 hours.

Excretion - urine and feces:

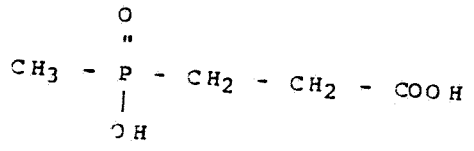
For males: "Urinary excretion amounted to 13.8 and 14.1% of the dose totally administered to dogs by daily treatments of 1 and 8 mg/kg respectively. The major part of radioactivity was excreted via the feces amounting to 83.5 and 82.0% for the low and high dose level, respectively. Additional radioactivity (1.1 - 1.2%) was found in the cage wash. Based on these findings, total recoveries after 96 hours...were 98.4 and 97.3% for the low and high dose level, respectively."

For females: "Excretion via the urine was 14.1 and 17.0% for the low and high dose level, respectively. Radioactivity appearing in the feces accounted for 80.2 and 78.8%, respectively. The cage wash ranged...from 2.2 to 1.5%.

It is noted that the above findings were from single dogs/sex/each dose level.

Refer to the appended copy of table 3 (p. 133) for percentage recoveries of radioactivity in urine, feces and cage wash.

Most of the material excreted in the urine was the parent compound, but a considerable amount (up to 24.7% in high-dose animals - see table 16, p. 146, appended page III) was the metabolite HOE 061517, with the following structure:



Organs and brain:

From table 11 (p. 141):

Level of radioactivity in organs and tissues of male and female dogs 6 hours after the first treatment with 14C-HOE 039866 (Values are from single dogs, and are expressed as mcg parent equivalents per g of fresh weight).

	Males			Females		
	Dose level - mg/kg/day					
Organ	0	1	8	0	1	8
Heart*	0.020	0.031	0.078	0.028	0.029	0.070
Liver, left lobe	0.018	0.055	0.274	0.018	0.045	0.210
Kidneys†	0.020	0.247	1.311	0.028	0.166	1.026
Cortex	0.015	0.025	0.050	0.017	0.024	0.048
Cerebellum	0.013	0.021	0.034	0.022	0.022	0.038
Midbrain	0.031	0.027	0.042	0.027	0.036	0.041
Brain stem	0.035	0.041	0.053	0.023	0.045	0.057
Spinal cord	0.018	0.029	0.136	0.022	0.027	0.050

*Means of values from left and right ventricles
†Means of values from left and right kidneys

From table 12 (p. 141):

Level of radioactivity in organs and tissues of male and female dogs 6 hours after the last treatment with 14C-HOE 039866 (Values are from single dogs, and are expressed as mcg parent equivalents per g of fresh weight).

	Males			Females		
	Dose level - mg/kg/day					
Organ	0	1	8	0	1	8
Heart*	0.021	0.034	0.117	0.020	0.036	0.173
Liver, left lobe	0.030	0.234	2.447	0.035	0.367	1.565
Kidneys†	0.028	0.338	3.705	0.027	0.540	4.195
Cortex	0.021	0.036	0.213	0.023	0.050	0.219
Cerebellum	0.018	0.042	0.258	0.025	0.057	0.248
Midbrain	0.030	0.035	0.573	0.024	0.081	0.240
Brain stem	0.028	0.050	0.418	0.037	0.103	0.455
Spinal cord	0.024	0.021	0.250	0.020	0.035	0.217

*Means of values from left and right ventricles
†Means of values from left and right kidneys

From table 13 (p. 143):

Level of radioactivity in organs and tissues of male and female dogs 24 hours after the last treatment with 14C-HOE 039866 (Values are from single dogs, and are expressed as mcg parent equivalents per g of fresh weight).

	Males			Females		
	Dose level - mg/kg/day					
Organ	0	1	8	0	1	8
Heart*	0.021	0.028	0.062	0.024	0.028	0.069
Liver, left lobe	0.032	0.638	3.575	0.021	0.422	3.267
Kidneys†	0.022	1.099	6.098	0.020	0.490	5.130
Cortex	0.017	0.059	0.200	0.021	0.045	0.187
Cerebellum	0.024	0.055	0.230	0.023	0.048	0.263
Midbrain	0.022	0.060	0.251	0.018	0.055	0.187
Brain stem	0.048	0.074	0.365	0.033	0.066	0.385
Spinal cord	0.029	0.048	0.168	0.012	0.043	0.133

*Means of values from left and right ventricles
†Means of values from left and right kidneys

From table 14 (p. 144):

Level of radioactivity in organs and tissues of male and female dogs 48 hours after the last treatment with 14C-HOE 039866 (Values are from single dogs, and are expressed as mcg parent equivalents per g of fresh weight).

	Males			Females		
	Dose level - mg/kg/day					
Organ	0	1	8	0	1	8
Heart*	0.024	0.035	0.059	0.023	0.041	0.061
Liver, left lobe	0.024	0.491	3.659	0.023	0.245	2.049
Kidneys†	0.021	0.480	3.081	0.024	0.411	2.029
Cortex	0.020	0.045	0.236	0.019	0.041	0.173
Cerebellum	0.025	0.049	0.299	0.021	0.048	0.255
Midbrain	0.029	0.067	0.238	0.032	0.059	0.334
Brain stem	0.057	0.090	0.260	0.051	0.090	0.364
Spinal cord	0.019	0.042	0.125	0.020	0.045	0.117

*Means of values from left and right ventricles
†Means of values from left and right kidneys

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From table 15 (p. 145):

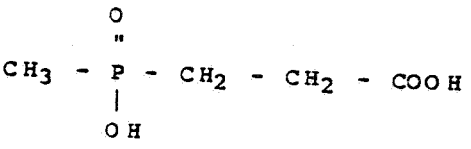
Level of radioactivity in organs and tissues of male and female dogs 96 hours after the last treatment with 14C-HOE 039866 (Values are from single dogs, and are expressed as mcg parent equivalents per g of fresh weight).

Organ	Males Dose level - mg/kg/day			Females		
	0	1	8	0	1	8
Heart*	0.031	0.031	0.047	0.026	0.037	0.051
Liver, left lobe	0.040	0.134	0.714	0.025	0.110	0.897
Kidneys†	0.024	0.121	0.811	0.024	0.103	1.258
Cortex	0.016	0.036	0.146	0.029	0.042	0.106
Cerebellum	0.024	0.050	0.235	0.025	0.054	0.139
Midbrain	0.032	0.040	0.246	0.044	0.066	0.181
Brain stem	0.049	0.084	0.355	0.056	0.103	0.206
Spinal cord	0.023	0.045	0.116	0.023	0.050	0.103

*Means of values from left and right ventricles
†Means of values from left and right kidneys

D. DISCUSSION:

The study demonstrates that when orally administered to dogs at 1 and 8 mg/kg/day most of the test material was eliminated unchanged via the feces, with about 14 to 17% excreted via the urine. Most of the material excreted in the urine was also the parent compound, but a considerable proportion (up to 24.7% in the 8 mg/kg/day dogs) was the metabolite HOE 061517, with the following structure:



This was the only metabolite that was detected.

The highest levels of retained radioactivity in dosed dogs were associated with the kidneys and liver.

It is reported (p. 14) that the "slight to moderate inhibition of cerebral and hepatic glutamine synthetase" was "due to competitive interference related to the structural analogy of the test article with glutamate... This enzyme inhibition was partly associated with a decrease of glutamine levels in the tissues and increased alpha-ketoglutarate levels in these organs. In respect to the decreased levels of phosphoethanolamine in some brain areas, it was not possible to assess the physiological relevance of this finding."

As a subchronic feeding study, this report is classified as core supplementary data. A subchronic feeding study (non-rodent) should usually be 90 days (rather than 28 days) and should preferably utilize at least 3 dose groups rather than the 2 which were present in this study. It is also noted that there was a lack of any significant toxicologic effect at the high dose level (8 mg/kg/day).

According to the first page of the report, the guidelines reference number for this study is 85-1. This corresponds to a metabolism study.

As a metabolism study, the study is classified acceptable, but it is noted that this study by itself would not fully meet Agency requirements for metabolism data if the active was to be used on food crops. However, the cover page indicates that the study is being submitted in support of the product's registration as a herbicide for non-food crop use.

Statistical Analysis

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The following statistical methods were used to analyze the body weights, food consumption, and clinical laboratory data.

Univariate one-way analysis of variance was used to assess the significance of intergroup differences.

If the variables could be assumed to follow a normal distribution, the Dunnett-test (many to one t-test) based on a pooled variance estimate was applied for the comparison between the treated groups and the control groups.

The Steel-test (many-one rank test) was applied when the data could not be assumed to follow a normal distribution.

Group means were calculated for continuous data and medians were calculated for discrete data (scores) in the summary tables.

Individual values, means, standard deviations and statistics were rounded off before printing. For example, test statistics were calculated on the basis of exact values for means and pooled variances and then rounded off to two decimal places. Therefore, two groups may display the same printed means for a given parameter, yet display different test statistics values.

References:

C.W. Dunnett: A Multiple Comparison Procedure for Comparing several Treatments with a Control, J. Amer. Statist. Assoc. 50, 1096-121 (1955).

R.G. Miller: Simultaneous Statistical Inference, Springer Verlag, New York (1981).

R.A. Fisher: Statistical Methods for Research Workers, Oliver and Boyd, Edinburgh (1950).

Table 3: Excretion pattern of male and female dogs repeatedly (10 x) treated with 14C-HOE 039866. (Values in % of the radioactivity totally applied.)

Exposure Time I	Exposure Time II	Males Dog - No. 7 Dose Level (mg/kg/day) 1	Males Dog - No. 13 Dose Level (mg/kg/day) 8	Females Dog - No. 25 Dose Level (mg/kg/day) 1	Females Dog - No. 31 Dose Level (mg/kg/day) 8
Urine 0		-	-	-	-
24		1.3	1.0	1.0	1.6
48		0.9	1.4	1.1	2.0
72		1.2	1.6	1.4	2.2
96		1.6	1.4	1.4	1.6
120		1.6	1.4	1.3	1.3
144		1.3	2.3	1.7	1.2
168		2.1	1.3	1.2	2.4
192		0.9	1.1	1.8	2.0
216	0	1.5	1.0	1.3	1.1
222	6	0.5	0.5	0.3	0.5
228	12	0.3	0.3	0.7	0.5
240	24	0.3	0.4	0.5	0.3
264	48	0.2	0.3	0.3	0.2
288	72	0.1	0.1	0.1	0.1
312	96	<0.1	<0.1	<0.1	<0.1
Subtotal		13.8	14.1	14.1	17.0
Feces 0		-	-	-	-
24		7.5	5.3	7.4	7.9
48		8.6	10.3	6.3	5.7
72		8.7	5.9	7.3	8.5
96		7.5	9.6	9.7	7.9
120		8.4	7.1	8.3	8.3
144		8.3	6.7	6.8	7.3
168		7.2	8.0	9.3	7.4
192		9.0	11.1	7.9	7.9
216	0	7.7	6.5	7.1	8.7
222	6	0.7	4.8	2.0	2.0
228	12	8.1	3.4	0.9	4.7
240	24	1.1	0.9	5.3	1.1
264	48	0.3	2.1	1.3	0.9
288	72	0.2	0.2	0.3	0.4
312	96	0.2	0.1	0.3	0.1
Subtotal		83.5	82.0	80.2	78.8
Cage Wash		1.1	1.2	2.2	1.5
Total		98.4	97.3	96.5	97.3

Legend: I = Time after starting the administration of 14C-HOE 039866
II = Time after the last administration of 14C-HOE 039866

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Table 16: Pattern of metabolites in urine excreted 0 - 48 hours after the last treatment with 14C-HOE 039866.
(Values in % of the radioactivity totally applied (I) and in % of the radioactivity recovered (II).)

	Dog - No.							
	7		13		25		31	
	Males				Females			
	Dose Level (mg/kg/day)							
	1		8		1		8	
	I	II	I	II	I	II	I	II
HOE 039866	2.8	100.0	1.9	75.3	2.8	88.8	2.1	79.3
HOE 061517	---	---	0.6	24.7	0.3	11.2	0.5	20.7
Total	2.8	100.0	2.5	100.0	3.1	100.0	2.6	100.0

Reviewed by: Whang Phang, Ph.D. 6/7/88
Section III, Tox. Branch (TS-769C)
Secondary reviewer: Marcia van Gemert, Ph.D. 6/7/88
Section III, Tox. Branch (TS-769C)

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DATA EVALUATION REPORT

STUDY TYPE: 28-Day Oral Dose Range Finding Study in Rats

CHEMICAL: HOE 039866; Monoammonium [2-amino-4-(hydroxymethyl-phosphinyl)butanoate]; Ignite®

ACCESSION NUMBER: 403456-07 CASWELL NO.: 580I

EPA ID NO: 8340-EO/8340-EI PROJECT NO.: 8-0146

SPONSOR: Hoechst Celanese Corp.

TESTING FACILITY: RCC, Research and Consulting Co. AG
P.O. Box CH-4452, Itingen, Switzerland

CITATION: Suter, P., Sachsse, K. et al. 28-Day Range Finding With HOE 039866 Technical in Rats. Research and Consulting Co. AG, Switzerland. Project No.: 018949; Report No.: A29425; Feb. 10, 1984. Submitted by Hoechst Celanese Corp.

CONCLUSIONS: When groups of Wistar rats (5/sex/dose) were fed HOE 039866 at dietary concentrations of 0, 50, 500, 2500, and 5000 ppm for 28 days, the following compound-related changes were observed:

1). At week 1 of treatment, decreased food intake was seen in 2500 and 5000 ppm males and females.

2). There was 11-20% increase in brain cholinesterase activity in all treated females relative to that of the controls.

3). A slight increase in urine specific gravity in all treated females. The increase was statistically significant in 50, 500, and 5000 ppm females.

4). There were increases in absolute and relative (kidney/body weight) kidney weights in all treated females.

This study provides useful information for understanding the toxicology of the test chemical. Based upon the increases in brain acetylcholinesterase activity and in absolute and relative kidney weights in all treated females, a NOEL for the toxicity of the test chemical can not be established. Since it is a dose-range finding study, it is classified as a Supplementary Study.

A. MATERIALS:

1. Test compound: Technical grade HOE 039866, a white crystalline powder with purity of 95.3%.

2. Test animals: Four to 5 weeks old Wistar KFM-HAN rats which weighed 72-113 gm (males) and 50-73 gm (females) were obtained from KFM Kleintierfarm Madoerin AG, Switzerland.

B. STUDY DESIGN:

1. Animal assignment

According to the report, animals were examined, acclimated for 7 days, and randomly assigned to the following test groups:

Test Group	Concentration in diet (ppm)	Number of animals male	female
1 Cont.	0	5	5
2	50	5	5
3	500	5	5
4	2500	5	5
5	5000	5	5

2. Diet preparation

The test chemical was mixed with diet to form pellets, and water was added to aid pelleting. The frequency of diet preparation was not specified in the report. Sample of the test diet were analyzed for stability and homogeneity of the test chemical.

The results indicated that the mean concentrations of the test chemical in the diet were 101.1%, 95.3%, 92.7% and 105.9% of the nominal concentrations of 50, 500, 2500, and 5000 ppm, respectively. The test chemical was found to be stable in the diet over 21 days at room temperature. Homogeneity of the test diet was within ±15%.

3. Animals received food and water ad libitum.

4. Statistics: The information on statistical methods applied in this study were excerpted from the submission and presented in Appendix 1.

5. Quality assurance was performed, and a signed statement was included in the report.

C. METHODS AND RESULTS:

1. Observations

Animals were inspected daily for signs of toxicity and mortality. No clinical signs for systemic toxicity or death were seen during the study.

2. Body Weight

Animals were weighed weekly for the duration of the study. The body weight data are presented in Table 1. There was no significant difference in body weights of treated animals relative to those of the controls.

3. Food consumption and compound intake

Food consumption was recorded weekly. The results are presented in Table 2.

At the first two weeks of the study, 2500 and 5000 ppm males and females consumed slightly less food than the controls.

No compound intake data were reported except that in the Summary of the report the author mentioned that 500 ppm was equivalent to 53 mg/kg/day for males and 58 mg/kg for females.

4. Ophthalmological examination data were not reported.

5. Blood was collected at the 4th week from all animals for hematology and clinical analyses. The CHECKED (X) parameters were examined.

a. Hematology

X	Hematocrit (HCT)*	X	Leukocyte differential count*
X	Hemoglobin (HGB)*	X	Mean corpuscular HGB (MCH)

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TABLE 1

BODY WEIGHT (GRAM)
SUMMARY

			0 PPM	50 PPM	500 PPM	2500 PPM	5000 PPM
FEMALES							
PRETEST							
DAY	1	MEAN	60	60	59	68	62
WEEK	1	ST.DEV	6.1	5.9	5.7	3.1	6.8
TREATMENT							
DAY	3	MEAN	102	105	103	109	100
WEEK	1	ST.DEV.	7.2	7.7	8.0	3.4	10.8
DAY	10	MEAN	129	132	128	133	123
WEEK	2	ST.DEV	9.4	8.6	9.2	6.0	13.8
DAY	17	MEAN	146	151	145	151	140
WEEK	3	ST.DEV	11.1	9.5	10.9	4.5	16.2
DAY	23	MEAN	164	166	161	166	155
WEEK	4	ST.DEV	11.5	10.9	13.1	4.5	18.0
MALES							
PRETEST							
DAY	1	MEAN	85	83	92	[illegible]	91
WEEK	1	ST.DEV	12.6	9.8	11.2	[illegible]	13.1
TREATMENT							
DAY	3	MEAN	143	140	150	[illegible]	144
WEEK	1	ST.DEV	17.3	14.7	[illegible]	[illegible]	18.4
DAY	10	MEAN	190	186	195	[illegible]	178
WEEK	2	ST.DEV	18.8	17.7	13.3	[illegible]	21.9
DAY	17	MEAN	228	223	228	[illegible]	215
WEEK	3	ST.DEV	19.0	21.8	15.5	[illegible]	24.1
DAY	23	MEAN	263	253	255	[illegible]	247
WEEK	4	ST.DEV	19.0	25.2	20.8	[illegible]	26.0

(DATA EXCERPTED FROM Submission)

TABLE 2

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FOOD CONSUMPTION (G/ANIMAL/DAY) SUMMARY

			0 PPM	50 PPM	500 PPM	2500 PPM	5000 PPM
MALES							
DAY 1		MEAN	21	18	19	15	16
WEEK 1		ST.DEV	---	---	---	---	---
DAY 8		MEAN	24	22	22	20	21
WEEK 2		ST.DEV	---	---	---	---	---
DAY 15		MEAN	25	23	22	22	22
WEEK 3		ST.DEV.	---	---	---	---	---
DAY 22		MEAN	26	24	23	23	24
WEEK 4		ST.DEV	---	---	---	---	---
FEMALES							
DAY 1		MEAN	14	14	13	12	11
WEEK 1		ST.DEV	---	---	---	---	---
DAY 8		MEAN	16	16	16	16	15
WEEK 2		ST.DEV.	---	---	---	---	---
DAY 15		MEAN	16	16	16	15	15
WEEK 3		ST.DEV	---	---	---	---	---
DAY 22		MEAN	17	17	17	17	17
WEEK 4		ST.DEV	---	---	---	---	---

(DATA EXCERPTED FROM Submission)

X		X	
x	Leukocyte count (WBC)*	x	Mean corpuscular HGB conc.(MCHC)
x	Erythrocyte count (RBC)*	x	Mean corpuscular volume (MCV)
x	Platelet count*	x	Reticulocyte count
	Blood Clotting Measurements		
x	(Thromboplastin time)		
	(Clotting time)		
x	(Prothrombin time)		

* Required for subchronic and chronic studies

The hematological findings are summarized in Table 3a and 3b. In males, MCV and MCH were significantly decreased in 500 and 5000 ppm groups. Reticulocytes were increased in 2500 and 5000 ppm groups, and the increase in 2500 ppm animals was statistically significant (Table 3a).

In females, the reticulocytes were significantly increased in 5000 ppm group (Table 3b). Other changes were small and sporadic.

b. Clinical Chemistry

X		X	
	Electrolytes:		Other:
x	Calcium*	x	Albumin*
x	Chloride*	x	Blood creatinine*
	Magnesium*	x	Blood urea nitrogen*
x	Phosphorous*	x	Cholesterol*
x	Potassium*	x	Globulins
x	Sodium*	x	Glucose*
	Enzymes	x	Total Bilirubin*
x	Alkaline phosphatase	x	Total Serum Protein*
x	Cholinesterase(blood & brain)		Triglycerides
	Creatinine phosphokinase*°	x	Serum protein electrophoresis
x	Lactic acid dehydrogenase		
x	Serum alanine aminotransferase (also SGPT)*		
x	Serum aspartate aminotransferase (also SGOT)*		
	gamma glutamyl transferase	x	LDH
	glutamate dehydrogenase		

* Required for subchronic and chronic studies
‡ Should be required for OP
° Not required for subchronic studies

Clinical biochemistry data for males and females are presented in Table 4a and 4b, respectively.

In male rats the following changes were observed (Table 4a):

- Slight increase in total bilirubin in all treated males, but statistical significance was only found in 5000 ppm group.

TABLE 3a

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HEMATOLOGY SUMMARY
MALES

	RBC T/l	HB mmol/l	HCT l/l	MCV fl	MCH fmol	MCHC mmol/l	PLATELETS G/l	RETIC %
AFTER 4 WEEKS								
0 PPM	8.6	9.8	0.49	56.6	1.14	20.0	1038	0.036
50 PPM	9.0	9.9	0.49	54.6	1.10	20.2	1016	0.036
500 PPM	9.1	9.8	0.48	52.8 **	1.08 *	20.4	1121	0.037
2500 PPM	8.6	9.7	0.48	55.6	1.12	20.1	1058	0.049 **
5000 PPM	9.1	9.7	0.49	54.0 *	1.07 *	19.8	1167	0.045

			DIFF. WBC COUNT (REL)					
	MEN /100 WBC	WBC G/l	BAND %	SEG. %	EO. %	BASO. %	LYMPH %	MONO %
AFTER 4 WEEKS								
0 PPM	0.0	6.8	0.00	0.16	0.01	0.00	0.78	0.04
50 PPM	0.6	6.9	0.00	0.14	0.01	0.00	0.82	0.03
500 PPM	0.4	6.2	0.00	0.16	0.02	0.00	0.79	0.03
2500 PPM	0.0	6.4	0.00	0.23	0.00	0.00	0.71	0.05
5000 PPM	0.3	6.8	0.00	0.20	0.01	0.00	0.75	0.05

	DIFF WBC COUNT (REL)		COAGULATION	
	PLAS %	OTHER %	PT SEC	PTT SEC
AFTER 4 WEEKS				
0 PPM	0.00	0.00	13.9	24.9
50 PPM	0.00	0.00	14.4	24.3
500 PPM	0.00	0.00	14.3	25.0
2500 PPM	0.00	0.00	14.2	23.7
5000 PPM	0.00	0.00	13.9	25.0

* / ** T-test based on pooled variance significant at 5% (*) or 1% (**) level

(DATA Excerpted From Submission

Table 36

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HEMATOLOGY
FEMALES

SUMMARY

	RBC T/l	HB mmol/l	HCT l/l	MCV fl	MCH fmol	MCHC mmol/l	PLATELETS G/l	RETIC %
AFTER 4 WEEKS								
0 PPM	8.6	9.3	0.46	54.0	1.09	20.1	1137	0.031
50 PPM	8.7	9.7	0.48	55.6	1.12	20.1	1107	0.036
500 PPM	9.0	9.7	0.48	53.6	1.08	20.3	1198	0.030
2500 PPM	9.0	9.6	0.48	53.8	1.08	20.1	1086	0.033
5000 PPM	8.3	9.3	0.47	56.3	1.12	20.1	1208	0.040 **

	DIFF. WBC COUNT (REL)							
	MEN /100 WBC	WBC G/l	BAND %	SEG. %	EO. %	BASO %	LYMPH. %	MONO. %
AFTER 4 WEEKS								
0 PPM	0.2	5.0	0.00	0.17	0.01	0.00	0.78	0.04
50 PPM	0.0	6.1	0.00	0.14	0.01	0.00	0.81	0.03
500 PPM	0.0	5.1	0.00	0.16	0.01	0.00	0.81	0.03
2500 PPM	0.4	5.3	0.00	0.12	0.01	0.00	0.85	0.02
5000 PPM	0.0	5.1	0.00	0.19	0.00	0.00	0.78	0.03

	DIFF WBC COUNT (REL)		COAGULATION	
	PLAS. %	OTHER %	PT SEC	PTT SEC
AFTER 4 WEEKS				
0 PPM	0.00	0.00	13.9	20.3
50 PPM	0.00	0.00	13.4	23.2 **
500 PPM	0.00	0.00	13.[illegible]	19.[illegible]
2500 PPM	0.00	0.00	13.5	21.[illegible]
5000 PPM	0.00	0.00	13.[illegible]	20.[illegible]

* , ** T-test based on pooled variance significant at 5% (*) or 1% (**) level

(DATA EXCERPTED From Submission)

- LDH levels was increased in all treatd groups, but only the lowest dose group showed any statistical significance. In addition, much fluctuation was present among the treatment groups.

- Erythrocyte acetylcholinesterase levels of all dosed males were increased relative to that of the controls. The increase was not dose-related, and statistical significance occurred in isolated groups (Table 4a).

- A slight decrease in calcium levels were seen in all treated males. Again the decrease was not dose-related.

- A significant drop in total protein was observed in 2500 and 5000 ppm males.

- With electrophoresis, many proteins were found to have changed relative to the controls. However, the changes presented much fluctuation, and most of these changes were relatively small.

The following changes were observed in <u>female rats</u> (Table 4b):

- Statistically significant decrease in urea levels were seen in 50, 2500, and 5000 ppm groups. The decrease was not dose-related.

- Slight but statistically significant increase in calcium was seen in all treated females, but the increase was not dose-related.

- Brain Acetylcholinesterase levels were increased in all treated females. The increases ranged from 11-20% of the controls, and statistical significance was seen in 50, 2500, and 5000 ppm groups (Table 4b).

- Several other statistically significant changes were also observed, but these changed were sporadic and showed large fluctuation among different treatment groups.

6. <u>Urinalysis</u>°

Urine samples were collected from 5 animals/sex/dose at "after 4 week" test period. The CHECKED (X) parameters were examined.

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

* Required for chronic studies
° Not required for subchronic studies

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Table 4a

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CLINICAL BIOCHEMISTRY SUMMARY
MALES

	GLUCOSE mmol/l	UREA mmol/l	CREATININE umol/l	BILI T umol/l	CHOLEST.T. mmol/l	ASAT(GOT) ukat/l	ALAT(GPT) ukat/l	LDH ukat/l
AFTER 4 WEEKS								
0 PPM	6.07	5.77	56	0.9	1.44	1.50	0.61	2.37
50 PPM	6.22	6.38	52	1.6	1.36	1.62	0.62	4.14 *
500 PPM	6.04	6.47	51	1.2	1.32	1.37	0.51	2.76
2500 PPM	5.96	5.68	47	1.3	1.25	1.46	0.45	3.76
5000 PPM	5.70	5.04	50	2.1 *	1.37	1.38	0.46	3.46

	ALP ukat/l	BuChE-PLAS umol-SH/ml	AChE-ERY. umol-SH/ml	CALCIUM mmol/l	PHOSPHORUS mmol/l	SODIUM mmol/l	POTASSIUM mmol/l	CHLORIDE mmol/l
AFTER 4 WEEKS								
0 PPM	5.00	0.44	1.77	2.43	2.39	139.3	3.94	97.1
50 PPM	5.15	0.43	2.03 *	2.35 *	2.18 *	140.9	3.88	101.0 *
500 PPM	5.00	0.41	2.16 **	2.38	2.27	141.4	3.89	98.6
2500 PPM	4.85	0.46	1.94	2.34 *	2.27	141.7 *	4.20 *	98.5
5000 PPM	4.31	0.46	2.00 *	2.36 *	2.42	140.4	4.02	98.4

	PROTEIN T g/l	PROT ELECTROPH (REL) ALBUMIN	A1-GLOB	A2-GLOB	SB-GLOB	G-GLOB	A/G RATIO
		1	1	1	1	1	
AFTER 4 WEEKS							
0 PPM	65.5	0.518	0.165	0.034	0.249	0.034	1.08
50 PPM	63.8	0.532	0.166	0.035	0.238	0.030	1.14
500 PPM	63.6	0.515	0.185 **	0.037	0.238	0.026	1.07
2500 PPM	61.8 **	0.524	0.180 **	0.042 *	0.224 **	0.030	1.10
5000 PPM	61.7 **	0.558 **	0.132 **	0.032	0.241	0.036	1.27 **

	PROT ELECTROPH (ABS) ALBUMIN g/l	A1-GLOB g/l	A2-GLOB g/l	SB-GLOB g/l	G-GLOB g/l	TISSUE ANALYSIS AChE-BRAIN umol-SH/g
AFTER 4 WEEKS						
0 PPM	33.9	10.8	2.2	16.3	2.2	4.56
50 PPM	33.9	10.6	2.2	15.2 *	1.9	3.95
500 PPM	32.9	11.8	2.3	15.1 *	1.6	4.06
2500 PPM	32.3	11.1	2.3	13.8 **	1.6	3.83
5000 PPM	34.5	9.1 **	2.0	14.9 *	2.2	4.54

* / ** T-test based on pooled variance significant at 5% (*) or 1% (**) level.

(DATA Excerpted From Submission)

Table 4b

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CLINICAL BIOCHEMISTRY SUMMARY
FEMALES

	GLUCOSE mmol/l	UREA mmol/l	CREATININE umol/l	BILI. T umol/l	CHOLEST T mmol/l	ASAT(GOT) ukat/l	ALAT(GPT) ukat/l	LDH ukat/l
AFTER 4 WEEKS								
0 PPM	5.90	9.02	56	1.4	1.22	1.38	0.36	2.91
50 PPM	5.52	6.78 *	57	1.5	1.15	1.44	0.46	6.12
500 PPM	5.62	7.17	53	1.9	1.32	1.67	0.45	14.78
2500 PPM	5.29 *	6.66 *	54	1.4	1.16	1.38	0.35	6.92
5000 PPM	5.82	6.44 **	53	1.8	1.25	1.35	0.44	3.61

	ALP ukat/l	BuChE-PLAS umol-SH/ml	AChE-ERY. umol-SH/ml	CALCIUM mmol/l	PHOSPHORUS mmol/l	SODIUM mmol/l	POTASSIUM mmol/l	CHLORIDE mmol/l
AFTER 4 WEEKS								
0 PPM	3.33	1.11	2.15	2.24	1.87	142.2	3.49	103.7
50 PPM	3.89	1.39	1.99	2.33 **	2.22 **	140.1	3.82	102.4
500 PPM	2.82	1.31	2.11	2.32 *	1.93	141.3	3.73	102.3
2500 PPM	2.36	1.50 *	2.20	2.34 **	2.00	135.7 **	3.95	98.3 *
5000 PPM	2.99	1.44	2.10	2.36 **	1.95	141.2	3.83	99.3

	PROTEIN T. g/l	PROT. ELECTROPH. (REL) ALBUMIN 1	A1-GLOB. 1	A2-GLOB. 1	SB-GLOB 1	G-GLOB 1	A/G RATIO
AFTER 4 WEEKS							
0 PPM	61.1	0.615	0.096	0.025	0.230	0.033	1.60
50 PPM	60.5	0.581 *	0.122 **	0.040 **	0.224	0.034	1.40 *
500 PPM	62.1	0.585 *	0.129 **	0.035 *	0.214	0.036	1.41 *
2500 PPM	64.2 *	0.608	0.098	0.032	0.226	0.035	1.56
5000 PPM	62.1	0.631	0.089	0.028	0.214	0.037	1.72

	PROT ELECTROPH (ABS) ALBUMIN g/l	A1-GLOB. g/l	A2-GLOB g/l	SB-GLOB g/l	G-GLOB g/l	TISSUE ANALYSIS AChE-BRAIN umol-SH/g
AFTER 4 WEEKS						
0 PPM	37.6	5.9	1.5	14.1	2.0	4.20
50 PPM	35.1 *	7.4 **	2.4 **	13.6	2.0	4.78 * (14%)
500 PPM	36.3	8.0 **	2.1 *	13.3	2.3	4.67 (11%)
2500 PPM	39.0	6.3	2.1 *	14.6	2.3	4.95 ** (18%)
5000 PPM	39.2	5.6	1.8	13.3	2.3	5.04 ** (20%)

* , ** T-Test based on pooled variance significant at 5% (*) or 1% (**) level.

(DATA Excerpted From Submission)

Urinalysis data are presented in Table 5. In treated males the parameters in urinalysis were comparable to those of the controls.

In females, there was a slight increase in specific gravity in all treatment groups relative to the controls. The increase was statistically significant in 50, 500, and 2500 ppm females, a dose-related response was not present.

7. Sacrifice and Pathology -

All animals that died and that were sacrificed on schedule were subject to gross pathological examination and the CHECKED (X) tissues were collected for histological examination. The (XX) organs in addition were weighed.

X		X		X	
	Digestive system		Cardiovasc./Hemat.		Neurologic
	Tongue		.Aorta*	x	.Brain*†
	.Salivary glands*		.Heart*		Periph. nerve*#
	.Esophagus*		.Bone marrow*		Spinal cord (3 levels)*#
	.Stomach*		.Lymph nodes*		.Pituitary*
	.Duodenum*	x	.Spleen*		Eyes (optic n.)*#
	.Jejunum*		.Thymus*		Glandular
	.Ileum*		Urogenital		.Adrenals*
	.Cecum*	xx	.Kidneys*†		Lacrimal gland#
	.Colon*		.Urinary bladder*		Mammary gland*#
	.Rectum*		.Testes*†		.Parathyroids*††
xx	.Liver*†		Epididymides		.Thyroids*††
	Gall bladder*#		Prostate		Other
	.Pancreas*		Seminal vesicle		Bone*#
	Respiratory		Ovaries*†		Skeletal muscle*#
	.Trachea*		.Uterus*		Skin*#
	.Lung*			x	All gross lesions and masses*
	Nose°				
	Pharynx°				
	Larynx°				

* Required for subchronic and chronic studies
° Required for chronic inhalation
In subchronic studies, examined only if indicated by signs of toxicity or target organ involvement
† Organ weights required in subchronic and chronic studies
†† Organ weight required for non-rodent studies

a. Organ Weight

The absolute and relative (organ/body weight) organ weight data are presented in Table 6.

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TABLE 5

URINALYSIS SUMMARY

MALES

	VOLUME/18h ml	SPEC.GRAV.	pH	PROTEIN SCORE 0/3	GLUCOSE SCORE 0/3	KETONE SCORE 0/3	BILIRUBIN SCORE 0/3	BLOOD SCORE 0/3	UROBILI SCORE 0/2
AFTER 4 WEEKS									
0 PPM	7.2	1.038	7	2	0	1	0	0	0
50 PPM	7.2	1.036	7	2	0	1	0	0	0
500 PPM	6.7	1.036	7	2	0	1	0	0	0
2500 PPM	7.1	1.035	7	2	0	1	0	0	0
5000 PPM	7.5	1.035	7	2	0	1	0	0	0

FEMALES

AFTER 4 WEEKS									
0 PPM	5.2	1.034	6	1	0	0	0	0	0
50 PPM	4.5	1.042 **	6	2	0	0	0	0	0
500 PPM	4.2	1.041 *	6	2	0	1	0	0	0
2500 PPM	4.2	1.040 *	6	2	0	0	0	0	0
5000 PPM	4.5	1.038	6	2	0	0	0	0	0

* ** T-Test based on pooled variance significant at 5% (*) or 1% (**) level

(DATA EXCERPTED FROM Submission)

-M-

TABLE 6

006936

ORGAN WEIGHTS (GRAM)
SUMMARY

MALES

		0 PPM	50 PPM	500 PPM	2500 PPM	5000 PPM
BODY W	MEAN	248.2	246.7	253.3	228.8	237.0
	ST. DEV.	16.5	23.7	17.9	8.8	28.3
LIVER	MEAN	8.46	7.86	7.84	7.31	7.47
	ST. DEV.	0.82	0.95	1.08	0.46	0.96
KIDNEYS	MEAN	1.81	1.80	1.89	1.87	1.88
	ST. DEV.	0.20	0.22	0.30	0.05	0.27

FEMALES

BODY W	MEAN	163.7	161.1	157.1	162.3	156.7
	ST. DEV	17.0	8.3	12.4	3.2	19.2
LIVER	MEAN	5.07	5.61	5.25	5.32	5.19
	ST. DEV.	0.57	0.37	0.90	0.41	0.36
KIDNEYS	MEAN	1.09	1.30 *	1.27	1.37 **	1.19
	ST. DEV.	0.14	0.09	0.22	0.10	0.16

* ** t-test significant at 5% (*) or 1% (**)

ORGAN TO BODY RATIOS (%)
SUMMARY

MALES

		0 PPM	50 PPM	500 PPM	2500 PPM	5000 PPM
LIVER	MEAN	3.407	3.182	3.096 *	3.199	3.149 *
	ST. DE	0.178	0.180	0.229	0.240	0.105
KIDNEYS	MEAN	0.727	0.728	0.744	0.820**	0.790
	ST. DE.	0.048	0.040	0.074	0.044	0.027

FEMALES

LIVER	MEAN	3.106	3.490 *	3.225	3.282	3.294
	ST. DE.	0.242	0.271	0.213	0.271	0.194
KIDNEYS	MEAN	0.667	0.810**	0.801**	0.842**	0.758 *
	ST. DE.	0.059	0.057	0.073	0.072	0.033

* ** t-test significant at 5% (*) or 1% (**)

(DATA EXCERPTED From Submission)

In males, the absolute organ weights of liver and kidney are comparable to those of the controls. Statistically significant increase in kidney to body weight was observed in 2500 ppm males; this increase could be attributed by the slight decrease of body weight in this group of animals relative to that of the controls.

In females, statistically significant increases in absolute kidney weight was seen in 50 and 2500 ppm groups and in kidney to body weight in all treated groups.

b. Gross Pathology

According to the report, slight reddening of the gastric mucosa was observed in 4/5 males of 5000 ppm group. Dark and red areas were also observed in 1/5 males of 50 ppm group. No other gross lesions were observed. However, the individual animal data on gross pathology were not reported, and the above results could not be verified.

c. Histopathology

Increased incidences of kidney tubular atrophy and calcification were observed in 50 ppm female relative to the controls (Table 7). However, these increases were not seen in animals of higher dose groups, and they were not compound related. However, the individual animal histopathology data were not reported, and the summary data presented in Table 7 could not be verified.

DISCUSSION

When groups of Wistar rats (5/sex/dose) were fed HOE 039866 at dietary concentrations of 0, 50, 500, 2500, and 5000 ppm for 28 days, the following changes in treated animals were observed:

1. At week 1 of treatment, decreased food intake was seen in 2500 and 5000 ppm males and females. Although this decrease was obvious, it did not show a statistically significant difference relative to the control and was not accompanied by a decrease in the body weight of these animals at the week 1.

2. There were many changes in clinical biochemistry parameters, but most of these changes presented much fluctuation among the treatment groups and essentially none had shown any dose-related response. However, there was a 11-20% increase in brain cholinesterase activity in all treated females relative to that of the controls.

3. A slight increase in urine specific gravity in all treated females. The increase was statistically significant in 50, 500, and 5000 ppm females.

006936

TABLE 7

PATHOLOGY REPORT
SUMMARY TABLES

PAGE PAT =
RCC-PROJECT 0:8465

TEST SUBSTANCE: HOE 039866 TECHNICAL
TEST SYSTEM : RAT, 28 DAYS, ORAL
SPONSOR : HOECHST AG, FRANKFURT/MAIN

EPS AG REF : 88042 HPA
DATE : 03-MAR-88
EPS AG PATHDATA SYSTEM

NUMBER OF ANIMALS WITH MICROSCOPIC FINDINGS BY ORGAN/GROUP/SEX (MC)

	DOSE GROUP	01		02		03		04	
	SEX	M	F	M	F	M	F	M	F
ORGAN/FINDING	NO. ANIMALS	5	5	5	5	5	5	5	5
KIDNEYS	NO. EXAM.	5	5	5	5	5	5	5	5
- HYDRONEPHROSIS				1			1		2
- CALCIFICATION			2		4		1		1
- PROTEINACEOUS CASTS									
- TUBULAR ATROPHY					4				

(DATA EXCERPTED From Submission)

4). There were increases in absolute and ralative (kidney/body weight) kidney weights in all treated females, and statistically significant increases were seen in 50 and 5000 ppm groups for absolute kidney weight and in all treated females for relative kidney weight.

This study provides useful information for understanding the toxicology of the test chemical. Based upon the the increases in brain acetylcholinesterase and in absolute and relative kidney weights in all treated females, a NOEL for the toxicity of the test chemical can not be established. Since this study is a dose range finding study, it is classified as a Supplementary Study.

It should be noted that the report should include individual animal data on histopathology.

Appendix 1

Information Excerpted from Submission

RIN 5218-93 / Tox Review for Glufosinate Log # 293

Page_____ is not included in this copy.

Pages 73 through 82 are not included.

The material not included contains the following type of information:

____ Identity of product inert ingredients.

____ Identity of product impurities.

____ Description of the product manufacturing process.

____ Description of quality control procedures.

____ Identity of the source of product ingredients.

____ Sales or other commercial/financial information.

____ A draft product label.

____ The product confidential statement of formula.

____ Information about a pending registration action.

✓ FIFRA registration data.

____ The document is a duplicate of page(s) _____.

____ The document is not responsive to the request.

The information not included is generally considered confidential by product registrants. If you have any questions, please contact the individual who prepared the response to your request.

Reviewed by: Whang Phang, Ph.D. 9/20/88 006936
Section II, Tox. Brnach (TS-769C)
Secondary reviewer: Marcia van Gemert, Ph.D. 9/26/88
Section II, Tox. Branch (TS-769C)

DATA EVALUATION REPORT

STUDY TYPE: 28-Day feeding study (rats)

CHEMICAL: HOE 039866; monoammonium [2-amino-4-(hydroxymethyl-phosphinyl)butanoate]; Ignite® (herbicide)

ACCESSION NUMBER: 403456-26 TOX. CHEM. NO.: 580I

EPA ID No: 8340-EO/8340-EI EPA PROJECT No: 8-0146

SPONSOR: Hoechst Celanese Corp.

TESTING LABORATORY: Hoechst AG, 6230 Frankfurt AM Main 80
Federal Republic of Germany

CITATION: Ebert, E., Leist, K. -H., Mayer, D., and Langer, K. -H. (1986). Hoe 039866 - Active ingredient technical (Code: 039866 OH ZD97 0001) study on the mode of action following subchronic oral administration (4-week feeding study) to Wistar rats. Pharma Research Toxicology and Pathology, Hoechst AG, Germany. Hoechst Report No.: A34553; Project No.: 772. Oct 28, 1986. Submitted by Hoechst Celanese Corp., Sommerville, NJ. Aug 27, 1987.

SUMMARY:
Groups of Wistar rats (40/sex) were administered Hoe 039866 at dietary concentrations of 0, 40, 200, 1000, and 5000 mg/kg diet for 28 days; the actual compound intakes expressed as mg/kg body weight/day were 0, 3.7, 18.7, 93.5, and 443. The treated animals were further divided into subgroups of 10 animals/sex. One subgroup from each dose was sacrificed at 0, 3, 7, and 28 days post treatment. The following effects were observed:
1). increased incidence of spontaneous activity, reactive behavior, and absence of pupillary reflex in 5000 mg/kg males,
2). decreased body weight and food consumption in 5000 mg/kg males and in both 5000 mg/kg males and females, respectively,
3). decreased dopamine level in the brain of highest dose females,
4). and decreased glutamine synthetase activity in the liver of animals which received 200 mg/kg or more, in the kidneys of males which received 200 mg/kg or more, and in the brain of 5000 mg/kg males.

Based upon decreased glutamine synthetase activity in liver and kidneys, the LOEL for Hoe 039866 was 18.7 mg/kg/day; NOEL, 3.7 mg/kg/day.

The report presented interesting information on the effects of Hoe 039866 on the behavioral and biochemial parameters. However, the study was not designed to meet the requirements for a subchronic toxicity study for reasons which include the following:
1). No hematology was carried out.

2). Histopathology was conducted only on liver, kidneys, and brain.
3). Clinical chemistry parameters were not examined.

This study is classified as supplementary.

A. MATERIALS:

1. Test compound: Hoe 039866 (technical grade); code No.: Hoe 039866 OH ZD97 0001; purity, 96.9%/

2. Test animals: Approximately 5 weeks (wks) old Wistar rats were obtained from Hoechst WISKf (SPF 71) breeding colony, Hoechst AG, Pharma Research Toxicology, Kastengrund. Male rats had a mean weight of 144 ± 7.8 gm; female rats, 131 ± 5.7 gm.

B. STUDY DESIGN:

1. Animal assignment

Animals were assigned randomly to the following test groups:

Test Group	Dose in diet (mg/kg diet)	Number of animals Male	female
1 Cont.	0	40	40
2	40	40	40
3	200	40	40
4	1000	40	40
5	5000	40	40

Each test group was further divided into a main group and 3 recovery groups which consisted of 10 animals/group. The animals in the recovery groups were sacrificed at days 3, 7, and 28 post treatment as indicated in the following:

Test Group	Main Group		Recovery Groups 1		2		3	
	Sacrifice (days post treatment) 0		3		7		28	
	M	F	M	F	M	F	M	F
1 (0 mg/kg diet)	10	10	10	10	10	10	10	10
2 (40 mg/kg ")	10	10	10	10	10	10	10	10
3 (200 mg/kg ")	10	10	10	10	10	10	10	10
4 (1000 mg/kg ")	10	10	10	10	10	10	10	10
5 (5000 mg/kg ")	10	10	10	10	10	10	10	10

2. Diet preparation

For each test concentration, 1 kg of premix was prepared. Subsequently, 1 kg of premix was blended into 39 kg of diet (Altromin 1321). The report did not indicate how the test diet was stored. Two samples were taken from each diet mix for content analysis.

The test diet was found to be stable for 30 days, and the administered concentrations corresponded to the nomial concentrations.

3. Animals received food and water ad libitum. Food consumption was determined twice weekly, and water consumption was measured once weekly as consumption / 100 gm animal body weight.

4. Special examinatoins of behavioral and neurological status

Comprehensive observational assessments of behavioral and neurological status were carried out according to the methods of S. Irwin, Psychopharmacologia 13. 222-257, (1968). The examinations, which included spontaneous behavior, behavioral reaction to various manipulations, reflexes, and autonomous clinical signs were carried out weekly on the animals in the main group throughout the treatment period and on recovery groups during the first 2 weeks of the recovery period. The methods of scoring are presented in the Appendix.

5. Biochemical studies:

The details of the procedures are presented in the Appendix.

A). Determination of catecholamines such as
-noradrenaline (NA),
-dopamine (DA), and
-dehydroxyphenyl acetic acid (DOPAC), a metabolite of DA
was performed in the brain tissue of 5 animals/sex.

B). Determination of glutamate synthetase activity in brain, liver, and kidneys was conducted on 5 animal/sex.

C). Determination of NH_4^+ levels in brain, liver, and kidneys was performed on 5 animals/sex.

D). Determination of free amino acids in brain, liver, and kidneys of 5 males/group was conducted. The following amino acids were measured:

-glutamine	(Gln)	
-glutamate	(Glu)	
-asparagine	(Asn)	
-aspartate	(Asp)	
-alanine	(Ala)	-liver only
-glycine	(Gly)	
-taurine	(Tau)	-brain only

E. Examinations of the liver-energy metabolism, biosynthesis of non-essential amino acids were also carried out. The following parameters were determined:

Energy metabolism: - isocitrate dehydrogenase (NADP-dep.)
- succinate dehydrogenase
- malate enzyme
- fructose diphosphatase
- glycogen

Glutathione metabolism: - glutathione
- gama-glutamyl transpeptidase

Biosynthesis of non-essential amino acids:
- glutamate pyruvate transaminase (GPT)
- glutamate oxalacetate transaminase (GOT)
- glutamate dehydrogenase (GlDE

5. Statistics: The Procedures section of the report did not indicate what statistical methods were used, but the individual experimental report showed the following tests were used:

-Dunnett test
-Parametric procedure of Sidak
-Distribution-free procedure of Nemenyi/Dunnett

RESULTS:

Mortality: The mortality rate of treated and control animals was similar.

Comphrehensive behavioral assessment: The behavioral assessment was conducted in the control, 1000, and 5000 mg/kg animals. In the controls and 1000 mg/kg rats, there was essentially no difference in the parameters examined (Table 1 A, B, C, & D). In 5000 mg/kg males, increased number of animals with spontaneous activity, catalepsia, reactive behavior, miosis, and absence of pupillary reflex was seen (Table 1 E). Incidence of miosis and absence of pupilary reflex was also found in 5000 mg/kg females (Table 1 F). There was a reduction in the body temperature of 5000 mg/kg males, but this change subsided at the end of the treatment period.

Body weight: The body weights of all treated females were comparable to those of the controls (Main group, Table 2). In 5000 mg/kg males, the body weight was slightly decreased during treatment, but after treatment the body weights of these animals slowly returned to the level of the controls (Recovery Group 3, Table 2).

Food consumption and compound intake: The food consumption in animals which received 1000 mg/kg test agent or less was comparable to that of the control rats. The food intake in 5000 mg/kg animals was slightly decreased (see the followig table). The compound intake by the test animals is also shown in the following table:

Main Food Consumption and Compound Intake

Dose group (mg/kg)	Males					Females				
	0	40	200	1000	5000	0	40	200	1000	5000
Food Consumption (g/100g bw./d)	9.5	9.3	9.3	9.3	8.9	9.2	9.1	9.2	8.9	8.5
Compound Intake (mg/kg/d)	--	3.7	18.7	93.5	443	--	3.7	18.3	89.4	424

* Data excerpted from page 22 of the report.

Hematology: No blood samples were collected for examination.

Clinical Chemistry: Data on clinical chemistry was not presented.

Sacrifice and Pathology: At sacrifice liver, brain, and kidneys were removed and weighed. Additional tissues were not examined.

Gross Pathology: No compound related gross changes were found in all treated animals.

Organ Weights: Among the three organs weighed, kidney weight of 5000 mg/kg females was ever so slightly increased relative to that of the control (control, 1.26 $\pm$ 0.09; 5000 mg/kg, 1.39 $\pm$ 0.07)(Table 2).

Microscopic Pathology: No microscopic pathology was carried out in this study.

Concentrations of Catecholamines in the Brain: In the Main Group, which was sacrificed at the end of the treatment period, highest dose females showed a statistically significant decrease in dopamine (NA) level, but this decrease did not persist as indicated by the measurements of the animals in the Recovery I Group (Table 3).

Glutamine Synthetase Activity (GS): The levels of GS in the liver of both males and females which received 200 mg/kg diet or more was significantly decreased (Table 4a, b, & c). This decrease was still evident at day 7 after treatment (Recovery Group 2).

In kidneys of the treated males a slight decrease in GS level was seen during the treatment, but this decrease did not show statistical significance until recovery period as indicated by the animals in 3 and 7 days recovery (Table 4A).

In the brain, statistically significany decrease in GS level was seen in 5000 mg/kg diet group males during treatment and at the 3 day recovery period (Table 4A).

NH_4^+ levels in brain, liver, and kidneys: Consistent and dose-related changes in NH_4^+ level were not seen in any organ examined (Table 5).

Amino acids in brain, liver, and kidneys: In the brain of male rats, no consistent changes in the levels of amino acids were detected (Table 6A). Sporadic changes in certain amino acid levels in the kidneys, but these changes did not show any dose-related reponse (Table 6B). In the liver, slight changes in certain amino acids were seen, but again they were not dose-related or consistent (Table 6C).

Energy and carbohydrate metabolism: The changes of the enzymes, isocitrate dehydrogenase, succinate dehydrogenase, malate enzyme, fructose diphosphatase or glycogen, which are involved in energy and carbohydrate metabolism, were slight and not dose-related (Table 7).

Glutathione metabolism: Glutathione contents in the liver of treated male and female rats were comparable to those of the controls (Table 8), and gamma-glutamyltranspeptidase level was slightly increased in highest dose males (Table 7). It should be noted that there appeared to be an inconsistency in the report of the level of gamma-glutamyl-transpeptidase between the data presented in Table 1.1.2 and that described in page 136 of the report. The data in Table 1.1.2 showed that this enzyme was elevated in highest dose males instead of in the females as indicated on page 136.

Biosynthesis of non-essential amino acids: To determine the effects of Hoe 039866 on the biosynthesis of certain amino acids (alanine & asparagine), the activities of the enzymes, glutamate oxalacetate transaminase (GOT) and glutamate pyruvate transaminase (GPT), were examined. The results indicated that GOT activities in both male and female livers of all dosed animals were comparable to those of the controls (Table 7). The GPT levels were significantly decreased in rats which received 200 mg/kg diet or more whereas in females this decrease was slight and only obser ed in 200 and 5000 mg/kg diet animals. The changes in GPT was not a dose-related response.

DISCUSSION

Groups of Wistar rats (40/sex) were administered Hoe 039866 at dietary concentrations of 0, 40, 200, 1000, and 5000 mg/kg diet for 28 days. The treated animals were further divided into subgroups of 10 animals/sex. One subgroup from each dose was sacrificed at 0, 3, 7, and 28 days post treatment.

The data indicated that the mortality rate of treated animals was comparable to that of the controls. The comprehensive observational assessment of neurological signs did not show much difference between the controls and the animals which received 1000 mg/kg diet or less. In 5000 mg/kg males, increased incidence of spontaneous activity, catalepsia, reactive behavior, and absence of pupilary reflex was seen.

During the treatment period decreased body weight was seen in 5000 mg/kg diet males. Decreased food consumption was observed in highest dose males and females.

A slight increase in mean kidney weight of 5000 mg/kg females was also seen.

Special biochemical examinations were carried out, and the following results were obtained:

1). Catecholamines: The dopamine level in the brain of the highest dose females was significantly decreased, but this change returned to the level of the controls during the post treatment period.

2). Glutamine synthetase activity (GS): The GS level in the liver of animals which received 200 mg/kg or more was significantly decreased. A slight drop in GS level in the kidneys of the treated males was also seen. The GS level in the brain of 5000 mg/kg males was significantly decreased.

3). NH_4^+ concentration: NH_4^+ level in brain, liver, and kidney of all treated animals was comparable to that of the controls.

4). Amino acids: Compound-related changes in amino acids of brain, liver, and kidneys were not seen.

5). Energy and carbohydrate metabolism: Hoe 039866 did not affect the enzymes involved in energy and carbohydrate metabolism.

6). Glutathione: The glutathione level in the liver of treated males and females was comparable to that of the controls.

7). Biosynthesis of non-essential amino acids: The effects of Hoe 039866 on the enzymes which were involved in the bio-

synthesis of non-essential amino acids were examined, and the results indicated that the test agent did not affect the activities of these enzymes.

The report presented interesting information on the effects of Hoe 039866 on the behavioral and biochemial parameters. However, the study was not designed to meet the requirements for a sub-chronic toxicity study for the following reasons:

1). No hematology was carried out.
2). Histopathology was conducted only on liver, kidneys, and brain.
3). Clinical chemistry parameters were not performed.

This study is classified as supplementary.

RIN 5218-93 / TOX Review for Glufosinate Log # 293

Page_____ is not included in this copy.

Pages 91 through 121 are not included.

The material not included contains the following type of information:

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Section III, Tox. Branch (TS-769C)
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DATA EVALUATION REPORT

STUDY TYPE: 13-Week feeding study in mice

CHEMICAL: HOE 039866; Monoammonium [2-amino-4-(hydroxymethyl-phosphinyl)butanoate]; Ignite®

ACCESSION NUMBER: 403456-09 CASWELL NO.: 580I

EPA ID NO: 8340-EO/8340-EI PROJECT NO: 8-0146

SPONSOR: Hoechst Celanese Corp.

TESTING FACILITY: RCC, Research and Consulting Co. AG
P.O. Box CH-4452, Itingen, Switzerland

CITATION: Suter, P., Horst, K. et al. 13-week oral toxicity (feeding) study with HOE 039866 technical in mice. Research and Consulting Co. AG, Switzerland. Project No.: 018516; April 29, 1986. Submitted by Hoechst Celanese Corp.

CONCLUSION

When groups of NMRI mice (10/sex/dose) were fed HOE 039866 at dietary concentrations of 0, 80, 320, and 1280 ppm for 13 weeks, the following compound-related effects were observed in treated mice:

1. Significant increases in serum aspartate aminotransferase and in alkaline phosphatase were seen in high dose males.

2. Increases in absolute and relative liver weights were seen in mid and high dose males.

Based upon the changes in clinical biochemistry and organ weights in mid and high dose males, LEL for HOE 039866 was established as 320 ppm; NOEL, 80 ppm. The study is classified as Core Minimum.

A. MATERIALS:

1. Test compound was HOE 039866 technical with purity of 95.3%, and the chemical was a white crystalline powder.

2. Test animals: 4 week old NMRI mice, which weighed 18-22 gm for males and 16-20 gm for females, were used in this study. These animals were obtained from KFM, Kleimtierfarm Madoerin AG, Switzerland.

B. STUDY DESIGN:

1. Animal assignment

Animals were acclimated for 7 days, and they were randomly assigned to the following test groups:

Test Group	Dose in diet (ppm)	13-week study male	female
1 Cont.	0	10	10
2 Low (LDT)	80	10	10
3 Mid (MDT)	320	10	10
4 High(HDT)	1280	10	10

2. Diet preparation

The test chemical was mixed with diet to form pellets. The frequency for preparing the treatment diet was not specified. Samples of the test diet were analyzed for stability and homogeneity of the test chemical.

The results indicated that the mean concentrations of the test chemical in the diet were 118.8%, 81.5%, and 91.5% of the targeted concentrations for groups 2, 3, and 4, respectively. The homogeneity varied from ±15% of the mean concentrations of the analyzed samples except for group 3 and 4 (week 12). The test chemical at levels of 50-5000 ppm was found to be stable for 21 days at room temperature.

3. Animals received food and water ad libitum.

4. Statistical methods used for analyzing the results of this study are presented in Appendix 1.

5. Quality assurance statement was included in the report.

C. METHODS AND RESULTS:

1. Observations

Animals were inspected twice daily for signs of toxicity and mortality. No signs of toxicity were reported in treated animals. Essentially all animals survived to the end of the study except one mid dose female which died during blood sampling.

2. Body Weight

Animals were weighed weekly. The mean body weights of treated mice were comparable to those of the controls (Table 1).

3. Food consumption

Food consumption was measured daily over a 7-day feeding period. In both treated males and females, there were slight increases in food consumption at various treatment periods and in different dose groups (Table 2). However, the "treatment means" were comparable between treated and control animals. In addition, the mean food conversion ratios were similar between treated and control mice during a major interval of the study.

Compound Intake

The mean of the weekly mean compound intake (mg/kg/day) was reported as follows:

	mg/kg/day		
Nominal Con.	80 ppm	320 ppm	1280 ppm
Males	16.55	67.14	277.70
Females	19.35	86.62	288.19

4. Ophthalmological examinations and hearing tests were conducted. In addition, teeth and mucous membranes of all test animals were also examined. No compound-related effects were observed in these examinations.

TABLE 1
Summary of Body Weight of HOE 039866 Treated & Control Mice

			MALES				FEMALES			
			0 PPM	80 PPM	320 PPM	1280 PPM	0 PPM	80 PPM	320 PPM	1280 PPM
DAY	3	MEAN	25	25	25	24	22	21	21	20
WEEK	1	ST DEV	1.3	1.5	1.4	1.4	2.1	1.9	1.6	1.9
DAY	10	MEAN	30	31	30	29	25	25	24	24
WEEK	2	ST DEV	1.9	2.0	1.2	1.7	2.1	2.2	2.4	1.6
DAY	16	MEAN	32	33	32	30	26	26	25	25
WEEK	3	ST DEV	2.4	2.3	1.1	2.0	2.0	2.0	2.1	1.9
DAY	24	MEAN	33	34	33	32	27	26	26	26
WEEK	4	ST DEV	2.2	2.3	1.6	1.8	2.0	2.4	2.7	1.9
DAY	31	MEAN	34	36	34	34	27	27	27	26
WEEK	5	ST DEV	3.1	2.6	1.4	1.8	2.2	2.8	3.0	2.2
DAY	38	MEAN	36	37	35	35	28	28	28	27
WEEK	6	ST DEV	3.1	2.8	1.3	2.5	2.4	2.9	3.1	1.9
DAY	44	MEAN	36	37	36	36	29	29	28	28
WEEK	7	ST DEV	3.4	3.4	1.2	2.4	2.7	2.6	2.9	2.1
DAY	51	MEAN	37	38	38	38	30	30	29	29
WEEK	8	ST DEV	3.3	3.2	2.0	3.2	2.8	4.0	3.4	2.7
DAY	58	MEAN	37	38	38	38	31	30	29	30
WEEK	9	ST DEV	3.6	2.9	1.9	3.3	2.5	3.6	3.7	2.5
DAY	65	MEAN	38	40	39	38	31	30	29	30
WEEK	10	ST DEV	3.6	3.0	2.3	3.7	2.8	3.9	3.8	2.6
DAY	73	MEAN	38	40	39	38	31	30	30	29
WEEK	11	ST DEV	4.1	3.3	2.6	3.9	3.6	3.4	3.7	2.6
DAY	79	MEAN	40	40	40	40	31	30	31	31
WEEK	12	ST DEV	4.4	3.0	2.8	3.6	3.0	3.1	3.6	2.8
DAY	86	MEAN	40	40	41	40	31	31	31	31
WEEK	13	ST DEV	4.4	3.2	2.6	3.5	3.4	3.6	3.8	2.3

(DATA EXCERPTED FROM 13-WEEK FEED STUDY IN MICE)

TABLE 2

FOOD CONSUMPTION FOR HOE 039866 TREATED + CONTROL MICE (13-WEEK FEEDING)

(gm/animal/day)

			MALES				FEMALES			
			0 PPM	80 PPM	320 PPM	1280 PPM	0 PPM	80 PPM	320 PPM	1280 PPM
DAY 1 / WEEK 1		MEAN	0.4	0.8	0.7	0.5	0.6	0.6	0.6	0.7
		ST DEV								
DAY 8 / WEEK 2		MEAN	1.3	1.6	1.0	0.9	0.8	0.8	0.8	0.9
		ST DEV								
DAY 15 / WEEK 3		MEAN	0.9	1.9	0.9	1.0	0.9	0.6	0.7	0.8
		ST DEV								
DAY 22 / WEEK 4		MEAN	0.5	1.9	0.9	1.0	0.8	0.6	1.2	1.9
		ST DEV								
DAY 29 / WEEK 5		MEAN	0.6	1.7	1.0	1.8	1.6	0.8	1.4*	2.4
		ST DEV								
DAY 36 / WEEK 6		MEAN	0.5	2.3	1.6*	1.8*	2.0	0.8	1.6	1.3
		ST DEV								
DAY 43 / WEEK 7		MEAN	0.8	2.7*	2.1*	1.7*	1.5	1.5	1.8*	1.6
		ST DEV								
DAY 50 / WEEK 8		MEAN	0.4	2.4*	2.1*	1.8*	1.5	1.0	1.7	1.3
		ST DEV								
DAY 57 / WEEK 9		MEAN	0.9	2.6	1.8	1.4	1.3	1.6	2.0*	1.3
		ST DEV								
DAY 64 / WEEK 10		MEAN	0.8	2.5*	1.8*	2.0*	1.4	1.6	1.8*	1.1
		ST DEV								
DAY 71 / WEEK 11		MEAN	0.9	1.7	0.8	1.3	1.5	2.1*	1.6	1.3
		ST DEV								
DAY 78 / WEEK 12		MEAN	1.0	0.8	0.7	1.6*	2.1	1.3	1.3	1.5
		ST DEV								
DAY 85 / WEEK 13		MEAN	0.7	1.4	0.9	1.5	2.3	1.6	1.2	1.8
		ST DEV								
TREATMENT MEAN			7	7	7	7	6	7	7	6

* : t-test significant at 5%

(DATA EXCERPTED from Submission)

5. Blood samples were collected from 10 non-fasted mice/sex/dose after 13 weeks of treatment for hematology and clinical analysis. The CHECKED (X) parameters were examined.

a. Hematology

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

* Required for subchronic and chronic studies

The hematology data are presented in Table 3.

In treated males, there were slight but statistically significant increases in hematocit in high dose and in white blood cell counts in mid and high dose relative to that of the controls. A significant increase in monocyte was also observed in high dose dose males.

In treated females, slight decreases in hematocrit and red blood cells in mid and high dose, an increase in MCH in high dose, and a decrease in segmented white blood cell in all dose were seen. However, this decrease in segmented white blood cells could be due to a high control mean because the individual animal data indicated a rather wide fluctuation in the values of control animals relative to those of the treated animals.

Although most of these hematological changes were statistically significant, they were rather small and could not be considered as compound-related.

b. Clinical Chemistry

X		X	
	Electrolytes:		Other:
	Calcium*	x	Albumin*
x	Chloride*	x	Blood creatinine*
	Magnesium*	x	Blood urea nitrogen*
	Phosphorous*	x	Cholesterol*
x	Potassium*		Globulins
x	Sodium*	x	Glucose*
	Enzymes	x	Total Bilirubin*
x	Alkaline phosphatase	x	Total Serum Protein*
	Cholinesterase‡		Triglycerides
	Creatinine phosphokinase*°		Serum protein electrophoresis
x	Lactic acid dehydrogenase		
x	Serum alanine aminotransferase (also SGPT)*		

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TABLE 3

HEMATOLOGY SUMMARY

MALES

	RBC T/l	HB mmol/l	HCT l/l	MCV fl	MCH fmol	MCHC mmol/l	PLATELETS G/l	RETIC. l
AFTER 13 WEEKS								
0 PPM	10.3	9.4	0.52	50.6	0.91	17.9	1218	0.033
80 PPM	10.5	9.1	0.53	50.4	0.87 *	17.3 *	1418 **	0.032
320 PPM	10.2	9.0	0.51	50.2	0.88	17.6	1313	0.035
1280 PPM	10.1	9.0	0.50 *	49.4	0.89	18.1	1304	0.038

DIFF. WBC COUNT (REL)

	NEW. /100 WBC	WBC G/l	BAND 1	SEG. 1	EO. 1	BASO. 1	LYMPH. 1	MONO. 1
AFTER 13 WEEKS								
0 PPM	0.0	5.4	0.00	0.14	0.02	0.00	0.82	0.02
80 PPM	0.0	4.2	0.00	0.16	0.02	0.00	0.80	0.03
320 PPM	0.0	3.0 *	0.00	0.16	0.00	0.00	0.80	0.04
1280 PPM	0.0	3.2 *	0.00	0.18	0.02	0.00	0.75	0.05 *

DIFF. WBC COUNT (REL) | RED CELL MORPHOLOGY

	PLAS. 1	OTHER 1	POLY. SCORE 1/3	ANIS. SCORE 1/3	H.-J. BOD. SCORE 1/3
AFTER 13 WEEKS					
0 PPM	0.00	0.00	---	---	X
80 PPM	0.00	0.00	---	---	X
320 PPM	0.00	0.00	---	---	X
1280 PPM	0.00	0.00	---	---	X

FEMALES

	RBC T/l	HB mmol/l	HCT l/l	MCV fl	MCH fmol	MCHC mmol/l	PLATELETS G/l	RETIC. l
AFTER 13 WEEKS								
0 PPM	10.6	9.5	0.52	49.2	0.90	18.3	1056	0.036
80 PPM	10.2	9.2	0.50	49.4	0.90	18.3	1142	0.033
320 PPM	10.0 *	9.0 *	0.50 *	49.4	0.90	18.3	1240 *	0.031
1280 PPM	9.9 *	9.3	0.50 *	50.4	0.94 **	18.7	1114	0.034

DIFF. WBC COUNT (REL)

	NEW /100 WBC	WBC G/l	BAND 1	SEG. 1	EO. 1	BASO. 1	LYMPH. 1	MONO. 1
AFTER 13 WEEKS								
0 PPM	0.0	2.9	0.00	0.09	0.01	0.00	0.88	0.02
80 PPM	0.0	2.9	0.00	0.05 *	0.02	0.00	0.91	0.01
320 PPM	0.0	3.9	0.00	0.05 *	0.01	0.00	0.79	0.02
1280 PPM	0.0	3.9	0.00	0.06 *	0.02	0.00	0.91	0.01

DIFF. WBC COUNT (REL) | RED CELL MORPHOLOGY

	PLAS 1	OTHER 1	POLY. SCORE 1/3	ANIS. SCORE 1/3	H.-J. BOD SCORE 1/3
AFTER 13 WEEKS					
0 PPM	0.00	0.00	X	X	X
80 PPM	0.00	0.00	X	X	X
320 PPM	0.00	0.00	---	---	X
1280 PPM	0.00	0.00	---	---	X

* / ** T-Test based on pooled variance significant at 5% (*) or 1% (**) level
X See individual results

(DATA EXCERPTED From Submission)
(13-Week Feeding Study with HOE 039866 IN MICE)

|x| Serum aspartate aminotransferase (also SGOT)*
|x| gamma glutamyl transferase
| | glutamate dehydrogenase

* Required for subchronic and chronic studies
\# Should be required for OP
° Not required for subchronic studies

In treated males, there were significant increases in serum aspartate aminotransferase in high dose and in potassium in mid and high dose. Other changes were sporadically observed in low or mid dose (Table 4).

In treated females, a slight increase in alkaline phosphatase was observed in high dose. Other small changes were observed in either low dose or mid dose and not in the high dose.

7. Sacrifice and Pathology -
All animals that died and that were sacrificed on schedule were subject to gross pathological examination and the CHECKED (X) tissues were collected for histological examination. The (XX) organs in addition were weighed.

X		X		X	
	Digestive system		Cardiovasc./Hemat.		Neurologic
x	Tongue	x	.Aorta*	x	.Brain*†
x	.Salivary glands*	xx	.Heart*	x	Periph. nerve*#
x	.Esophagus*	x	.Bone marrow*	x	Spinal cord (3 levels)*#
x	.Stomach*	x	.Lymph nodes*	x	.Pituitary*
x	.Duodenum*	xx	.Spleen*	x	Eyes (optic n.)*#
x	.Jejunum*	xx	.Thymus*		Glandular
	.Ileum*		Urogenital	xx	.Adrenals*
x	.Cecum*	xx	.Kidneys*†		Lacrimal gland#
x	.Colon*	x	.Urinary bladder*	x	Mammary gland*#
	.Rectum*	xx	.Testes*†	x	.Parathyroids*††
xx	.Liver*†	x	Epididymides	xx	.Thyroids*††
x	Gall bladder*#	x	Prostate		Other
x	.Pancreas*	x	Seminal vesicle	x	Bone*#
	Respiratory	xx	Ovaries*†	x	Skeletal muscle*#
	.Trachea*	x	.Uterus*	x	Skin*#
x	.Lung*			x	All gross lesions and masses*
	Nose°				
	Pharynx°				
	Larynx°				

* Required for subchronic and chronic studies
° Required for chronic inhalation
\# In subchronic studies, examined only if indicated by signs of toxicity or target organ involvement
† Organ weights required in subchronic and chronic studies
†† Organ weight required for non-rodent studies

TABLE 4

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CLINICAL BIOCHEMISTRY
SUMMARY

MALES

	GLUCOSE mmol/l	UREA mmol/l	CREATININE umol/l	BILI. T. umol/l	CHOLEST.T. mmol/l	ASAT(GOT) ukat/l	ALAT(GPT) ukat/l	LDH ukat/l
AFTER 13 WEEKS								
0 PPM	10.54	10.23	32	5.6	3.69	1.08	0.70	9.18
80 PPM	10.44	9.64	37	5.2	3.39	1.25	0.65	7.65
320 PPM	9.87	9.83	34	4.5	3.59	1.01	0.77	8.49
1280 PPM	11.17	9.27	33	5.0	3.76	1.45 *	0.59	9.55

	ALP ukat/l	G-GT ukat/l	SODIUM mmol/l	POTASSIUM mmol/l	CHLORIDE mmol/l	ALBUMIN g/l	PROTEIN T. g/l
AFTER 13 WEEKS							
0 PPM	2.61	58.68	144.4	4.35	115.1	18.9	55.1
80 PPM	2.74	31.26 *	149.2 *	4.40	115.9	19.1	54.4
320 PPM	2.58	50.01	147.2	5.16 *	117.2	18.4	58.5
1280 PPM	2.42	47.79	143.1	5.39 *	110.5	18.4	53.8

FEMALES

	GLUCOSE mmol/l	UREA mmol/l	CREATININE umol/l	BILI. T. umol/l	CHOLEST.T. mmol/l	ASAT(GOT) ukat/l	ALAT(GPT) ukat/l	LDH ukat/l
AFTER 13 WEEKS								
0 PPM	11.15	7.39	41	6.0	2.41	1.25	0.75	7.97
80 PPM	10.28	7.53	31 *	6.9	2.92	2.21 *	0.88	8.51
320 PPM	9.98	7.58	34	5.9	2.42	1.64	1.22	10.80
1280 PPM	9.99	7.21	35	4.4	2.68	1.52	0.57	7.76

	ALP ukat/l	G-GT ukat/l	SODIUM mmol/l	POTASSIUM mmol/l	CHLORIDE mmol/l	ALBUMIN g/l	PROTEIN T. g/l
AFTER 13 WEEKS							
0 PPM	3.43	44.01	147.2	4.14	115.2	21 6	53.7
80 PPM	3.79	48.34	144.7	4.18	117.0	22.3	57.1
320 PPM	3.70	49.01	143.1 *	4.01	118.0	18.4 *	50.9
1280 PPM	4.72 *	56.01	144.3	4.62	117.7	20.2	53.3

* / ** : T-Test based on pooled variance significant at 5% (*) or 1% (**) level

(DATA EXCERPTED From Submission)
(13-Week Mouse Feeding Study with HOE 039866)

a. Organ weight

In treated males, absolute and relative liver weights (liver/body and liver/brain) were increased in mid and high dose males relative to those of the controls (Table 5a, 5b, & 5c). Although the increases in absolute liver weight did not show statistical significance, the increase in serum aspartate aminotransferase in combination with increases in absolute and relative liver weights indicated a compound-related effect. Other organ weights were comparable between treated and control animals.

b. Gross pathology

No summary of gross observations was reported, but the individual animal data did not indicate increased adverse effects in any organ of any dose group.

c. Histopathology

Histopathology findings are presented in Table 6. No treatment-related increase in any lesions was observed.

DISCUSSION

Groups of NMRI mice (10/sex/dose) were fed HOE 039866 at dietary concentrations of 0, 80, 320, and 1280 ppm for 13 weeks. The following changes in treated mice were observed:

1. Slight increase in food consumption at various intervals in both treated males and females was found.

2. Changes in hematology parameters were seen, and these changes were rather small and were not considered to be compound related.

3. Significant increase in serum aspartate aminotransferase and slight increase in alkaline phosphatase were seen in high dose males and females, respectively.

4. Increases in absolute and relative liver weights were seen in mid and high dose males.

Based upon the changes in clinical biochemistry and organ weights in mid and high dose males, LEL for HOE 039866 was established as 320 ppm; NOEL, 80 ppm. The study is classified as Core Minimum.

RIN 5218-93 / TOX Review for Glufosinate Log #293

Page_____ is not included in this copy.

Pages 132 through 137 are not included.

The material not included contains the following type of information:

____ Identity of product inert ingredients.

____ Identity of product impurities.

____ Description of the product manufacturing process.

____ Description of quality control procedures.

____ Identity of the source of product ingredients.

____ Sales or other commercial/financial information.

____ A draft product label.

____ The product confidential statement of formula.

____/ Information about a pending registration action.

____ FIFRA registration data.

____ The document is a duplicate of page(s) _____.

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006936

EPA: 68-02-4225
DYNAMAC No. 343-G
April 11, 1988

006936

DATA EVALUATION RECORD

IGNITE (HOE 039866)

Subchronic Dermal Toxicity Study in Rats

APPROVED BY:

Robert J. Weir, Ph.D.
Acting Department Manager
Dynamac Corporation

Signature: Robert J. Weir

Date: 4/12/88

006936

EPA: 68-02-4225
DYNAMAC No. 343-G
April 11, 1988

DATA EVALUATION RECORD

IGNITE (HOE 039866)

Subchronic Dermal Toxicity Study in Rats

REVIEWED BY:

Margaret E. Brower, Ph.D.
Principal Reviewer
Dynamac Corporation

Signature: Margaret E. Brower
Date: April 12, 1988

William L. McLellan, Ph.D.
Independent Reviewer
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Signature: Marcia van Gemert
Date: 5/10/88

DATA EVALUATION REPORT

TOX. CHEM. NO.:
MRID NO.:

STUDY TYPE: Subchronic dermal toxicity study in rats.

ACCESSION NUMBER: 403456-05.

TEST MATERIAL: HOE 039866, active ingredient technical.

SYNONYM: Ignite.

STUDY NUMBER: 83.0159, report No. A31477, project No. 84.0563.

SPONSOR: Hoechst Celanese Corporation, Somerville, NJ.

TESTING FACILITY: Hoechst AG, Federal Republic of Germany.

TITLE OF REPORT: HOE 039866-Active Ingredient Technical (CODE: HOE 039866 OH ZD95 0001) Testing for Subchronic Dermal Toxicity in Wistar Rats (21 applications over 30 days).

AUTHOR(S): Ebert, E. and Kramer, M.

REPORT ISSUED: May 10, 1985.

CONCLUSIONS:

Under the conditions of the study, dermal irritation was not found to occur on rats after repeated dermal exposure to HOE 039866 at dosage levels of 100, 300, or 1000 mg/kg/day, 5 days/week, for 21 days. A recovery study was conducted for 14 days following the 30-day dosing period. Two of 6 males dosed with 300 mg/kg/day and 4 of 11 males and 2 of 11 females dosed with 1000 mg/kg/day displayed aggressive behavior, piloerection, and a high startle response. There were no effects of toxicologic importance on body weights, food consumption, hematology, clinical chemistry, urinalysis, organ weights, or gross or microscopic pathology. Based on clinical observations, the LOEL is 300 mg/kg/day and the NOEL is 100 mg/kg/day.

Classification: This study report has certain inconsistencies, but these are not considered to appreciably influence the experimental results. The study, therefore, classified as Core Minimum.

A. MATERIALS:

1. Test Compound: HOE 039866, active ingredient technical; description: white crystalline hydroscopic powder; code No. HOE 039866 OH ZD95 0001; purity: 95.3%.

2. Test Animals: Species: rat; strain: HOE: Wiskf (SPF 71); age: 8-10 weeks; weight: males--143 to 154 g, females 144 to 154 g; source: Hoechst AG, Pharma Forschung Toxikologie Kastengrund breeding colony.

B. STUDY DESIGN:

1. Animal Assignment: Following 8 to 9 days of acclimation, animals were assigned to the following test groups using a computer-generated randomization scheme:

Test Group	Dosage Level (mg/kg/day)	Dosage Volume (mL/kg/day)	Main Study (21 days) Males	Main Study (21 days) Females	Recovery Study[a] (44 days) Males	Recovery Study[a] (44 days) Females
1 Control	0	1.3	6	6	5	5
2 Low (LDT)	100	1.3	6	6		
3 Mid (MDT)	300	1.3	6	6		
4 High (HDT)	1000	1.3	6	6	5	5

[a]The recovery study was conducted for 14 days following the 21-day dosing period (5 days/week for 30 days = 21 daily applications).

Animals were housed in an environmentally controlled room with a 12-hour light/dark cycle.

2. Dose Preparation: HOE 039866 technical was reported to be stored in the dark at 4°C. The test material was added to deionized water and topically applied in 7.5, 22.5, or 75% solutions (w/v) for the 100-, 300- or 1000- mg/kg/day dosage groups, respectively. The frequency of solution preparation and storage conditions were not reported. Homogeneity, test material concentration, and stability analyses of the administered solutions were not reported.

3. Preparation of Animal Skin: Prior to dosing, the hair was removed from the nape of the neck (approximately 10% of the total body surface) of each animal by clipping. The test material, in a volume of 1.3 mL/kg, was applied to the clipped area once per day, 5 days/week, for a total of 21 applications in 30 days. The area was covered and wrapped with an occlusive bandage for 6 hours, after which time the test site was washed with warm water. Control animals were treated with the vehicle (physiological saline) only.

4. Food and Water Consumption: Animals received food [Altromin-R 1324 pellets (Altromin GmbH Lage/Lippe)] and water ad libitum except for the period in which animals were kept in diuresis cages.

5. Statistics: Body weights were analyzed using the method of Sidak and the T-test if parametric procedures were indicated and the method of Nemenyi/Sidak if nonparametric procedures were indicated. Clinical pathology and organ weights were analyzed using the method of Sidak and Dunnett's test for parametric procedures and the methods of Nemenyi/Dunnett and Nemenyi/Sidak for nonparametric procedures. In addition, the T-test and Wilcoxon test were also utilized for clinical chemistry and organ weight determinations.

6. Quality Assurance Statement: A quality assurance statement was dated May 10, 1985, but not signed.

C. METHODS AND RESULTS:

1. Observations: Animals were inspected twice daily 5 days per week and once daily on weekends for mortality and signs of overt toxicity. Weekly examinations were conducted to determine neurological disturbances, opacity of the eyes, damage of the oral mucosa, and impairment of dental growth. Dermal evaluations were conducted prior to each topical application of the test compound.

Results: One high-dose male (animal No. 24) was sacrificed in a moribund condition on study day 16 after food refusal for 9 days; encrusted blood was found around the eyes, nose, and mouth. All other animals survived the study period. Table 1 presents clinical findings of animals observed during the dosing and recovery periods. Two males receiving 300 mg/kg/day displayed aggressive behavior; in addition, one of these males displayed piloerection, spasms, and convulsive jumping at dosing termination.[1] Four males and two females receiving 1000 mg/kg displayed aggressive behavior, piloerection, and a high startle response.

Dermal findings are presented in Table 2. Signs of slight dermal irritation were found in control and dosed females only on study days 7 and 8. No other dermal irritation was observed in dosed males or females throughout the study.

2. Body Weight: Rats were weighed at study initiation and twice weekly thereafter until study termination.

   Results: Representative body weight data are presented in Table 3. It was reported that no significant ($p < 0.05$) differences were noted between dosed and control groups. Mean body weights of high-dose males were slightly decreased during the recovery phase of the study; body weights of other males were similar to controls throughout the study. The body weights of dosed females were slightly increased when compared to concurrent controls throughout the study.

3. Food Consumption and Compound Intake: Food consumption was determined at the same intervals as body weights. Water consumption was determined once weekly for a 16-hour period during dosing and at recovery termination.

   Results: Food and water consumption were comparable in dosed and control males and females.

4. Ophthalmological Examinations: Ophthalmological examinations were not conducted.

5. Hematology and Clinical Chemistry: Blood was collected via the retrobulbar venous plexus at the end of the dosing period for hematological analyses from nonfasted males and females. Clinical analyses were conducted from blood collected from the vena cava cranialis.

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[1]Data as indicated in individual results.

TABLE 1. Summary of Clinical Findings[a] in Rats Dosed Dermally with HOE 039866 for 30 Days and Rats Observed For the 14-Day Recovery Period

	Males/Dosage Group (mg/kg/day)				Females/Dosage Group (mg/kg/day)			
Clinical findings	0	100	300	1000	0	100	300	1000
No symptoms	11/11	6/6	4/6	5/11	9/11	6/6	6/6	9/11
Mortality	0/11	0/6	0/6	1/11	0/11	0/6	0/6	0/11
Lesion due to removal of occlusive bandage by study animal	--[b]	--	--	--	2/11	--	--	--
Aggressive behavior	--	--	2/6	4/11	--	--	--	--
Kangaroo position	--	--	1/6	1/11	--	--	--	1/11
Piloerection	--	--	1/6	2/11	--	--	--	1/11
Convulsive jumping	--	--	1/6	--	--	--	--	--
Easily startled	--	--	1/6	3/11	--	--	--	--
Decreased respiratory rate	--	--	--	1/11	--	--	--	--
Hyperactivity	--	--	--	--	--	--	--	1/11
Lesion on left foreleg	--	--	--	1/11	--	--	--	--

[a] Number of animals with findings/dose group.

[b] No findings.

TABLE 2. Skin Irritation Findings[a] Observed on Days 7 and 8 in Female Rats Dosed Dermally with HOE 039866 for 21 Days

	Day 7/ Dosage Group (mg/kg/day)				Day 8/ Dosage Group (mg/kg/day)			
	0	100	300	1000	0	100	300	1000
Findings								
Erythema[b]	3/11	3/6	2/6	1/11	0/11	2/6	1/6	1/11
Dry, chapped skin	3/11	4/6	2/6	3/6	--[c]	--	--	--
Fine-scaled skin	3/11	2/6	--	3/6	--	1/6	1/6	1/11
Coarse-scaled skin	--	1/6	1/6	--	1/11	1/6	--	--
Scaling off	--	1/6	--	--	--	--	--	--

[a]Number of animals with findings/dosage group.

[b]Erythema scores for all dosages were 1 to 2, minimally perceptible.

[c]No findings.

TABLE 3. Representative Body Weight (g) Data from Rats Dosed Dermally with HOE 039866 for 21 Days and Observed for Either 30 or 44 Days

Dosage Group[a] (mg/kg/day)	Mean Body Weight (± SD) at Day 1	16	27	30	37	44
Males						
0	197 ± 10	267 ± 17	310 ± 19	311 ± 19	347 ± 13	372 ± 12
100	205 ± 12	270 ± 7	314 ± 5	309 ± 8	—[b]	—[b]
300	199 ± 2	254 ± 10	298 ± 38	286 ± 39	—[b]	—[b]
1000	200 ± 9	242 ± 41	300 ± 16	298 ± 15	322 ± 14*	353 ± 16
Females						
0	175 ± 6	191 ± 13	196 ± 12	198 ± 14	210 ± 12[c]	223 ± 9
100	181 ± 4	198 ± 8	215 ± 10	213 ± 10	—[b]	—[b]
300	180 ± 13	203 ± 17	218 ± 23*	214 ± 23	—[b]	—[b]
1000	179 ± 9	196 ± 16	215 ± 17*	209 ± 21	218 ± 25	224 ± 24

[a] Controls and high-dose groups contained 11 rats; low- and mid-dose groups contained 6 rats.

[b] All animals in low- and mid-dose groups and six rats in each of the control and high-dose groups were sacrificed at day 30.

[c] Female weights were determined on day 36.

* Significantly different from control values at $p < 0.05$ as evaluated by the study authors.

The checked (X) parameters were examined:

a. Hematology

X Hematocrit (HCT)†
X Hemoglobin (HGB)†
X Leukocyte count (WBC)†
X Erythrocyte count (RBC)†
X Platelet count†
X Reticulocytes
X Methemoglobin[a]
X Leukocyte differential count
X Mean corpuscular HGB (MCH)
X Mean corpuscular HGB concentration (MCHC)
X Mean corpuscular volume (MCV)
X Erythrocyte sedimentation rate
X Thromboplastin time
X Activated partial thromboplastin time
X Coagulation time

Results: Leukocyte values and activated partial thromboplastin time (APTT) were found to be slightly but significantly ($p < 0.05$) decreased in males receiving 300 mg/kg/day when compared to concurrent controls; however, these changes were not found in other dosed males or females and were considered by the authors to be of no toxicological significance since the differences were minimal and there was no dose-related trend. All other hematological parameters were similar in dosed and control males and females. Hematological determinations were not conducted following the recovery period since no compound-related changes were found during the dosing period.

b. Clinical Chemistry[b]

Electrolytes
X Calcium†
X Chloride†
Magnesium†
X Phosphorus†
X Potassium†
X Sodium†

Enzymes
X Alkaline phosphatase (ALP)
Cholinesterase
Creatinine phosphokinase†
X Lactic acid dehydrogenase
X Serum alanine aminotransferase (SGPT)†
X Serum aspartate aminotransferase (SGOT)†

Other
X Albumin†
X Albumin/globulin ratio
X Blood creatinine†
X Blood urea nitrogen†
X Cholesterol†
X Globulins
X Glucose†
X Total bilirubin†
X Direct bilirubin
X Total protein†
Triglycerides
X Uric acid
X Total lipids
X Serum electrophoresis

† Recommended by Subdivision F (October 1982) Guidelines.

[a] Methemoglobin was not evaluated for low- and mid-dose males and females since this finding was normal among high-dose animals.

[b] Based on clinical chemistry changes during the dosing period; only chloride and uric acid determinations were conducted in dosed males and sodium, potassium, and chloride determinations were conducted in dosed females following the recovery period.

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Results: It was reported that there were no toxicologically important effects on clinical chemistry parameters related to dosing. Following the dosing period, slight changes were found sporadically among electrolytes (sodium, potassium, chloride), uric acid, and globulins. However, these changes were minimal, not dose related, and reported to be within the range of normal biological variation (historical laboratory control levels were not reported.) Following the recovery period, all values were similar in dosed and control males and females.

6. Urinalysis: Urine was collected from fasted animals in diuresis cages following 20 topical applications of the test material at 28-29 days. The checked (X) parameters were examined:

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

Results: There were no effects of dosing on any urinary parameter. Urinary determinations were not conducted following the recovery period since no compound-related changes were found during the dosing period.

7. Sacrifice and Pathology: All animals that died and six animals/sex receiving 0, 100, 300, or 1000 mg/kg/day were sacrificed at 30 days and were subject to gross pathological examination; the checked (X) tissues were collected for histological examination. The (XX) organs from all animals were also weighed. Five control and high-dose males and females were observed for an additional 14-day recovery period prior to necropsy.

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† Recommended by Subdivision F (October 1982) Guidelines.

	Digestive system		Cardiovasc./Hemat.		Neurologic
	Tongue		Aorta†	XX	Brain†
	Salivary glands†	XX	Heart†		Peripheral nerve (sciatic nerve)†
	Esophagus†	X	Bone marrow†		Spinal cord (3 levels)
X	Stomach†		Lymph nodes†	XX	Pituitary†
	Duodenum†	XX	Spleen†	X	Eyes (optic nerve)†
X	Jejunum†	X	Thymus†		
	Ileum†				Glandular
	Cecum†		Urogenital	XX	Adrenals†
X	Colon†	XX	Kidneys†		Lacrimal gland
	Rectum†	X	Urinary bladder†		Mammary gland†
XX	Liver†	XX	Testes†		Parathyroids†
	Gallbladder†	X	Epididymides	XX	Thyroids†
X	Pancreas†	X	Prostate		
		X	Seminal vesicle		Other
	Respiratory	XX	Ovaries		Bone (sternum)†
	Trachea†	X	Uterus†		Skeletal muscle†
XX	Lung†			X	Skin (treated and untreated)
					All gross lesions and masses

Results:

a. Organ Weights: Absolute and relative organ weights of dosed males and females were similar to those of concurrent controls with the exception of the slight but significant ($p < 0.05$) decrease in relative brain weights of dosed females (control, 0.979 ± .073g; low dose, 0.887 ± 0.039g; mid dose, 0.885 ± 0.082g, high dose, 0.885 ± 0.045g). However, these decreases were due to the slight increases in body weight for these animals and were not considered to be compound related (control, 193 ± 15g, low dose, 213 ± 10g; mid dose, 214 ± 23g; high dose, 210 ± 13g).

b. Gross Pathology: Macroscopic examination of the test animals revealed no effects of dosing.

c. Microscopic Pathology: There were no histologic findings that were considered related to compound administration. It was reported that the dermis and epidermis of treated skin were slightly thicker with fewer hair follicles when compared to untreated skin. No obvious cause of severe illness could be found histologically for the one high-dose male that was sacrificed in a moribund condition on day 16.

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†Recommended by Subdivision F (October 1982) Guidelines.

D. STUDY AUTHORS' CONCLUSIONS:

The authors concluded that there were no effects of toxicological importance when HOE 039866 technical was administered dermally to rats in dosages of 100, 300, or 1000 mg/kg/day. Based on dermal irritation results, the authors classified the test material as a nonirritant. Based on minor clinical changes in animal behavior, the NOEL was determined to be 100 mg/kg/day.

E. REVIEWERS' DISCUSSION AND INTERPRETATION OF RESULTS:

The study design was adequate and complete, and the conduct of the study was adequate; however, food consumption was not statistically analyzed, and analyses of test material stability and homogeneity were not conducted. No individual clinical findings were reported for one high-dose female (animal No. 62) for study days 19 to 30. There was some discrepancy in data reported by the study authors. Individual dermal results (CBI study report pp. 333-374) indicated that signs of slight irritation were found on study days 7 and 8; however, this irritation was reported on days 5 and 6 in the discussion of dermal results (CBI study report p. 18). The test material was reported to be added to deionized water in order to obtain proper dosing solutions; however, control animals were reported to have been dosed with physiological saline as the test vehicle. Only one mid-dose male was indicated to have displayed aggressive behavior in the observation summary results of the study report; however; two mid-dose males were indicated to have displayed this behavior in the individual data results. Some histological tissues were reported to be missing; there was no summary tabulation of gross or histological examinations.

We agree with the study authors' conclusion that HOE 039866 technical can be classified as a nonirritant and, with the exception of some display of aggressive behavior in mid- and high-dose animals, there were no effects of toxicological importance following dermal administration of the test material. Based on clinical observation, the LOEL is 300 mg/kg/day and the NOEL is 100 mg/kg/day.

CONFIDENTIAL [illegible] INFORMATION
DOES [illegible]
NATIONAL SECURITY INFORMATION [illegible]

006936 006936

EPA: 68-02-4225
DYNAMAC No. 343-J
June 21, 1988

DATA EVALUATION RECORD

IGNITE

Subchronic Inhalation Toxicity Study in Rats

APPROVED BY:

Robert J. Weir, Ph.D.
Acting Department Manager
Dynamac Corporation

Signature: Sharon Ambrose for
Date: June 21, 1988

EPA: 68-02-4225
DYNAMAC No. 343-J
June 21, 1988

DATA EVALUATION RECORD

IGNITE

Subchronic Inhalation Toxicity Study in Rats

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Marcia Van Gemert, Ph.D.
EPA Section Head
Section V (TS-769C)

Signature: Marcia Van Gemert
Date: 6/27/88

DATA EVALUATION REPORT

STUDY TYPE: Subchronic inhalation toxicity study in rats.

ACCESSION NUMBER: 403456-06.

TEST MATERIAL: HOE 039866.

SYNONYM: Ignite.

STUDY NUMBER: 83.0160.

SPONSOR: Hoechst Celanese Corporation, Somerville, NJ.

TESTING FACILITY: Hoechst AG, Federal Republic of Germany.

TITLE OF REPORT: Testing for Subchronic Inhalation Toxicity--28 Exposures in 40 Days in SPF Wistar Rats.

AUTHOR(S): Hollander H., and Kramer, M.

REPORT ISSUED: March 26, 1985.

CONCLUSIONS:

Groups of 15 male and 15 female Wistar rats were exposed (nose only) to 0, 8, 20, or 46 mg/m^3 of HOE 039866 for 28 days over a period of 40 days. Of these, five male and five female rats were held without further exposure for an additional 29 days after the exposure phase. Two male and two female rats exposed to 46 mg/m^3 died during the study. Males exposed to 46 mg/m^3 and females exposed to 20 or 46 mg/m^3 gained more weight and consumed more food than controls. A decrease in thromboplastin time in males exposed to 8 or 46 mg/m^3 and an increase in coagulation time in females exposed to 20 or 46 mg/m^3 were noted. Slight but significant changes in several clinical chemistry parameters were noted; however, all values were within the normal range for this species. Organ weight changes were noted for the lung, brain, testes, pituitary, and spleen; however, these were within normal variation for this species. No gross or microscopic pathology was noted for any animals except those that died on study. These animals exhibited signs of severe stress.

The NOEL for rats is 8 mg/m^3.

Classification: Core Supplementary.

A. MATERIALS:

1. Test Compound: HOE 039866, active ingredient, technical; description: white powder; batch No.: OH ZC95 0001; purity: 95.3%.

2. Test Animals: Species: rat; strain: Wistar; age: 7-8 weeks; weight: males--155 to 186 g, females--151 to 172 g; source: Hoechst AG.

B. STUDY DESIGN:

1. Animal Assignment: Animals were assigned randomly to the following test groups:

Test Group	Target Exposure Concentration (mg/m^3)	Exposure Phase (40 days)		Recovery Phase (29 days)	
		Males	Females	Males	Females
1 Control	0	10	10	5	5
2 Low (LDT)	12	10	10	5	5
3 Mid (MDT)	25	10	10	5	5
4 High (HDT)	50	10	10	5	5

2. Dust Generation: The inhalation chambers were operated under dynamic conditions and the dust was dispersed using a "Wright Dust Feed" generator. Airflow through the generator was 1000 L/hour and tne dust concentration was regulated using differing gear ratios. The rats were exposed by nose only. Gravimetric measurements were conducted daily at 30 minutes and at 2 and 4 hours after exposure initiation. Analytical measurements were conducted on days 5, 12, 19, 26, 33, and 40 of the study. Particle size analysis was conducted once daily. The results are as follows:

Results:

Concentration (mg/m^3)		
Target	Gravimetric	Analytical
0	--	--
12	13	8
25	27	20
50	49	46

Based on the analytical results, rats were exposed to 0, 8, 20, or 46 mg/m^3, 6 hours/day, on 28 exposure days during a period of 40 days.

The mass median particle diameter was not reported; however, 75% of the particles (by weight) were less than 7 micrometers in diameter.

3. Food and Water Consumption: Animals received food (Altromin 1324 pellets) and water ad libitum except during exposure.

4. Statistics: The following procedures were utilized in analyzing the numerical data: Dunnett test, Sidak test, Student's t test, Wilcoxon test, and the distributed-free method of Nemenyi.

5. Quality Assurance: A quality assurance statement was signed and dated 8/11/87.

C. METHODS AND RESULTS:

1. Observations: Animals were inspected twice daily for signs of toxicity and mortality.

Results: Two males and two females exposed to 46 mg/m^3 died during the study. The two females died during the second exposure, one male died shortly after the second exposure, and one male died after the 17th exposure. The incidence of clinical signs is given in Table 1. Tono-clonic convulsions were noted in 8 of 15 males and 4 of 15 females exposed to 46 mg/m^3 as well as in 2 of 15 males exposed to 20 mg/m^3.

2. Body Weight: Animals were weighed twice weekly throughout the study.

Results: Mean body weights for males and females exposed to 46 mg/m^3 and females exposed to 20 mg/m^3 were as much as 14% higher (statistically significant at $p < 0.05$) than for control animals (Table 2).

3. Food Consumption: Consumption was determined and mean daily diet consumption was calculated.

Results: There was a slight increase in relative food consumption in males and females exposed to 46 mg/m^3. These increases were not significantly different from control values (Table 3).

4. Ophthalmology: Ophthalmological examinations were not performed.

TABLE 1. The Incidence of Clinical Signs in Rats Exposed to HOE 039866 for 28 Days

	Incidence During Exposure Phase (10 animals)							
	Males/Exposure (mg/m^3)				Females/Exposure (mg/m^3)			
Clinical Signs	0	8	20	46	0	8	20	46
Squatting position	0	1	3	6	0	0	0	4
Pilo-erection	0	1	2	4	0	0	0	2
Staggering gait	0	0	0	5	0	0	1	2
Aggressiveness	0	0	1	0	0	0	0	0
Hematuria	0	0	1	1	0	0	0	0
Tremors	0	0	0	0	0	0	1	1
Bite injuries	0	0	2	5	0	0	0	0
Weak postural reflex	0	0	0	2	0	0	0	0
Opacity of cornea	0	0	0	0	0	0	0	1
Jerky respiration	0	0	0	3	0	0	0	0
Narrowed eye openings	0	0	1	6	0	0	0	2
Contracted flanks	0	0	1	0	0	0	0	0
Hyperactivity	0	0	0	0	0	0	0	1
Salivation	0	0	1	2	0	0	0	1
Tono-clonic convulsions	0	0	2	8	0	0	0	4

TABLE 2. Selected Body Weight Values for Rats Exposed to HOE 039866 for 28 Days

Exposure Concentration (mg/m^3)	Group Mean Body Weight (g) at Day						
	1	5	12	26	41	54/56	69
Males							
0	197 ± 8	209 ± 12	229 ± 15	264 ± 18	285 ± 20	336 ± 10	369 ± 15
8	192 ± 8	205 ± 8	223 ± 11	257 ± 12	279 ± 16	331 ± 14	363 ± 18
20	193 ± 8	207 ± 10	224 ± 16	270 ± 21	300 ± 20	368 ± 33	400 ± 41
43	194 ± 6	195 ± 21	229 ± 16	265 ± 32	307 ± 17*	367 ± 21	402 ± 25
Females							
0	174 ± 4	180 ± 4	188 ± 5	197 ± 8	205 ± 8	217 ± 10	216 ± 11
8	178 ± 5	182 ± 6	189 ± 8	201 ± 10	210 ± 14	215 ± 17	219 ± 14
20	178 ± 8	183 ± 7	194 ± 9	213 ± 17*	222 ± 16*	236 ± 20	245 ± 23*
43	174 ± 6	173 ± 22	197 ± 7	216 ± 10*	228 ± 9*	243 ± 10	248 ± 9*

*Significantly different from control value ($p \leq 0.05$).

TABLE 3. Selected Food Consumption Data for Rats Exposed to HOE 039866 for 28 Days

Exposure Concentration (mg/m^3)	Group Mean Food Consumed (g/day) at Day					
	1	5	12	26	41	Complete Study
Males						
0	21.6	21.5	21.4	22.7	22.5	23.5
8	21.5	20.5	21.4	22.6	22.0	23.1
20	21.5	21.3	22.5	23.9	25.3	25.3
43	21.1	17.2	23.7	24.3	26.4	25.4
Females						
0	16.9	17.2	17.3	16.8	17.7	17.2
8	17.5	16.9	17.6	17.2	17.5	16.7
20	17.5	16.5	19.5	19.2	18.9	18.6
43	17.1	14.5	20.7	18.8	18.5	18.9

5. Hematology and Clinical Chemistry: Blood was collected 1 day following the final exposure, at the end of the exposure phase, and after the recovery phase for hematology and clinical 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 corpuscular HGB conc. (MCHC)
X	Erythrocyte count (RBC)†	X	Mean corpuscular volume (MCV)
X	Platelet count† (thrombocytes)	X	Coagulation time
	Reticulocytes[a]	X	Heinz bodies[a]
		X	Thromboplastin time
		X	Activated partial thromboplastin time

Results: There was a statistically significant decrease in thromboplastin time in males exposed to 8 mg/m^3 at the end of the exposure phase. This decrease was resolved during the recovery phase; however, at the end of the recovery phase, males exposed to 46 mg/m^3 exhibited a decrease in thromboplastin time. Females exposed to 20 mg/m^3 exhibited an increased coagulation time at the end of the exposure phase. At the end of the recovery phase, females exposed to 20 or 46 mg/m^3 exhibited an increase in reticulocytes. The study authors considered these changes to be toxicologically irrelevant since they were within normal ranges for this strain of rat and were not related to exposure concentrations.

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[a]Following the termination of exposure and recovery, reticulocyte and Heinz bodies were examined from controls and rats exposed to 46 mg/m^3 only; since significant differences were found in reticulocyte counts following recovery, all dosed males and females were examined for this parameter following recovery.

†Recommended by Subdivision F (October 1982) Guidelines.

b. Clinical Chemistry

	Electrolytes		Other
X	Calcium†		Albumin†
X	Chloride†	X	Blood creatinine†
	Magnesium†	X	Blood urea nitrogen†
X	Phosphorus†	X	Cholesterol†
X	Potassium†		Globulins
X	Sodium†	X	Glucose†
		X	Total bilirubin†
	Enzymes	X	Total protein†
X	Alkaline phosphatase (ALP)	X	Triglycerides
	Cholinesterase	X	Total lipids
	Creatinine phosphokinase†	X	Methemoglobin
X	Lactic acid dehydrogenase	X	Direct bilirubin
X	Serum alanine aminotransferase (SGPT)†	X	Electrophoresis
X	Serum aspartate aminotransferase (SGOT)†		

Results: At the end of the exposure phase of the study, one or more of the exposure groups exhibited decreased chloride, triglyceride, sodium, calcium, or protein concentrations; increased phosphorous or cholesterol concentration; or increased lactic acid dehydrogenase activity. All of these changes were resolved during the recovery phase; however, at the end of the recovery phase, one or more groups exhibited an increase in sodium, chloride, urea nitrogen, or triglyceride concentration or a decrease in cholesterol or total lipid concentration. In addition, at the end of the recovery phase, there was an increase in alpha-3 globulin in males exposed to 46 mg/m^3 and an increase in alpha-1 globulin in females exposed to 18 or 20 $mg/m.^3$ The study authors considered these changes to be toxicologically irrelevant since they were within normal limits for this strain of rat and were not related to exposure concentrations.

6. Urinalysis: Urine was not collected.

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†Recommended by Subdivision F (October 1982) Guidelines.

7. Sacrifice and Pathology: All animals that died or were sacrificed on schedule were subject to gross pathological examination, and the checked (X) tissues were collected for histological examination. In addition, the (XX) organs were weighed:

	Digestive system		Cardiovasc./Hemat.		Neurologic
	Tongue	X	Aorta†	XX	Brain†
X	Salivary glands†	XX	Heart†	X	Peripheral nerve (sciatic nerve)†
X	Esophagus†	X	Bone marrow†	X	Spinal cord (3 levels)
X	Stomach†	X	Lymph nodes†	XX	Pituitary†
X	Duodenum†	XX	Spleen†	X	Eyes (optic nerve)†
X	Jejunum†	X	Thymus†		
X	Ileum†				
X	Cecum†		Urogenital		Glandular
X	Colon†	XX	Kidneys†	XX	Adrenals†
X	Rectum†	X	Urinary bladder†		Lacrimal gland
XX	Liver†	XX	Testes†		Mammary gland†
	Gallbladder†	X	Epididymides		Parathyroids†
X	Pancreas†	X	Prostate	XX	Thyroids†
		XX	Seminal vesicle		
	Respiratory	XX	Ovaries		Other
X	Trachea†	X	Uterus†		Bone †
XX	Lung†			X	Skeletal muscle†
X	Nose with turbinalia				Skin
					All gross lesions and masses
				X	Thymus
				X	Diaphragm

Results:

a. Organ Weights: At the end of the exposure phase, there was a statistically significant decrease in relative lung weight in males exposed to 20 mg/m,^{3} in relative testes weight in males exposed to 46 mg/m,^{3} and in relative brain weight of females exposed to 20 mg/m^3; furthermore, there was an increase in relative brain weight and pituitary weight in females exposed to 46 mg/m^3 (Table 4). These differences were resolved during the recovery phase; however, at the end of the recovery phase, there was a decrease in relative brain weight in males exposed to 20 or 46 mg/m^3 and in relative spleen weight in females exposed to 8 mg/m^3. These relative weights were within the normal variation for this species.

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†Recommended by Subdivision F (October 1982) Guidelines.

TABLE 4. Selected Organ Weight Data for Rats Exposed to Hoe 039866 for 28 Days

Exposure Concentration (mg/m^3)	Group Mean Organ Weight at the end of Exposure and Recovery Phases					
	Exposure Phase				Recovery Phase	
	Lung		Testes		Pituitary	
	Absolute (g)	Relative (%)	Absolute (g)	Relative (%)	Absolute (mg)	Relative (% x 100)
Males						
0	1.41 ± 0.19	0.49 ± 0.9	3.02 ± 0.35	1.05 ± 0.08	10.4 ± 2.0	0.28 ± 0.05
8	1.30 ± 0.15	0.47 ± 0.04	2.79 ± 0.35	1.00 ± 0.10	11.2 ± 1.8	0.31 ± 0.04
20	1.25 ± 0.15	0.42 ± 0.04*	3.07 ± 0.09	1.04 ± 0.09	11.2 ± 1.0	0.28 ± 0.04
43	1.42 ± 0.27	0.47 ± 0.10	2.87 ± 0.19	0.94 ± 0.05*	13.3 ± 0.8	0.33 ± 0.02*

	Exposure Phase				Recovery Phase	
	Brain		Pituitary		Spleen	
	Absolute (g)	Relative (%)	Absolute (mg)	Relative (% x 100)	Absolute (g)	Relative (%)
Females						
0	1.90 ± 0.12	0.92 ± 0.06	11.8 ± 1.5	0.58 ± 0.2	0.46 ± 0.06	0.21 ± 0.03
8	1.88 ± 0.10	0.89 ± 0.04	11.4 ± 0.5	0.54 ± 0.03	0.37 ± 0.02	0.17 ± 0.05*
20	1.88 ± 0.14	0.86 ± 0.07*	11.9 ± 1.9	0.54 ± 0.07	0.46 ± 0.09	0.19 ± 0.03
43	1.94 ± 0.10	0.85 ± 0.05*	11.1 ± 1.9	0.49 ± 0.09*	0.45 ± 0.07	0.18 ± 0.03

*Significantly different from control value ($p \leq 0.05$).

b. Gross Pathology: No remarkable changes in gross pathology were noted during this study.

c. Microscopic Pathology

1) Nonneoplastic: No remarkable histopathological changes were noted in the animals that survived exposure. Of the four animals that died, one died from aspiration pneumonia and the other three showed signs of severe stress, i.e., cell atrophy in the thymus and bone marrow and contraction of the spleen. In addition, blood congestion and focal necrosis of the liver were found in two of these animals.

2) Neoplastic: No neoplasms were observed in this 28-day inhalation study.

D. STUDY AUTHORS' CONCLUSIONS:

Male and female rats exposed to a target concentration of 12 mg/m^3 of HOE 039866 for 28 days during a period of 40 days exhibited no remarkable toxicological signs or results. Rats exposed to target concentrations of 25 or 50 mg/m^3 of HOE 039866 exhibited marked exposure-related effects. Two males and two females exposed to 50 mg/m^3 died during the study. Based on these results, the NOEL and LOEL for rats exposed to HOE 039866 are 12 and 25 mg/m^3, respectively.

E. REVIEWERS' DISCUSSION AND INTERPRETATION OF RESULTS:

This study was purported to satisfy the subchronic toxicity requirements as stated in 40 CFR 158.135 for EPA guideline 82-4; however, the study consisted of 28 exposures in 40 days rather than the required 65 exposures in 90 days. Exposure levels were low and a target organ was not identified. According to the study authors, the NOEL for rats exposed to HOE 039866 was 12 mg/m^3 (or the analytical concentration of 8 mg/m^3). This value is considered to be tentative and may suffice until higher exposure levels are tested for the full 90 days to explore some of the variations noted in hematology (thromboplastin and coagulation times) and in relative organ weights (brain, lung, testes, pituitary, and spleen). The significance of increased body weight gain with increasing exposure concentration should be evaluated. [redacted]. In view of the hematological changes and the hematuria noted in clinical observations, a careful examination of hematology effects along with a complete urinalysis should be conducted in the 90-day study. As a matter of record, the study provides supplementary data, but does not identify a target organ and cannot be used to set exposure levels for a chronic study.

006936

EPA: 68-02-4225
DYNAMAC No. 343-H
May 17, 1988

DATA EVALUATION RECORD

IGNITE (HOE 039866)

Chronic Toxicity Feeding Study in Dogs

APPROVED BY:

Robert J. Weir, Ph.D.
Acting Department Manager
Dynamac Corporation

Signature: Robert J. Weir

Date: 5/17/88

006936

EPA: 68-02-4225
DYNAMAC No. 343-H
May 17, 1988

DATA EVALUATION RECORD

IGNITE (HOE 039866)

Chronic Toxicity Feeding Study in Dogs

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Principal Reviewer
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Date: May 17, 1988

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DATA EVALUATION REPORT

STUDY TYPE: Chronic toxicity feeding study in dogs.

ACCESSION NUMBER: 403456-08.

TEST MATERIAL: HOE 039866.

SYNONYM: Ignite.

STUDY NUMBER(S): A29827, project No. 019203.

SPONSOR: Hoechst Celanese Corporation, Somerville, NJ.

TESTING FACILITY: RCC, Research and Consulting Company AG, Intingen, Switzerland.

TITLE OF REPORT: HOE 039866 Substance Technical Grade (Code: HOE 039866 O H ZC95 0001) 12-Month Oral Toxicity (Feeding) Study in Beagle Dogs and Stability and Homogeneity Study in Dog Feed.

AUTHOR(S): Bathe, R., Frei, Th., Luetkemeir, H., Guenard, J., Ellgenausen, H., Chevalier, J., Terrier, Ch., Sachsse, K., and Sinnreich, D.

REPORT ISSUED: November 27, 1984.

CONCLUSIONS:

When HOE 039866 technical was fed to male and female beagle dogs for 12 months in the diet at levels of 2.0, 5.0, or 8.5 mg/kg/day, there were no overt signs of toxicity or dose-related effects on body weight, food consumption, ophthalmology, hematology, clinical chemistry, urinalyses, or organ weights. Two dogs receiving 8.5 mg/kg/day died during the study as a result of heart and circulatory system failure from rapid diet consumption; pathologies of these animals revealed multiple myocardial necrosis and necrotizing aspiration pneumonia. Electrocardiogram results of dosed males and females indicated a dose-related decrease in heart rate at 6 months; heart rates of dosed animals at 12-months were considered to be normal. Based on mortality, the systemic LOEL is 8.5 mg/kg/day; the NOEL is 5.0 mg/kg/day.

Classification: Core minimum.

A. MATERIALS:

1. Test Compound: HOE 039866 technical; description: white crystalline hydroscopic powder; batch No.: 0 H ZC95 0001; purity: 95.3%.

2. Test Animals: Species: dog; strain: beagle; age: 4-6 months; weight: males--4.2 to 8.0 kg, females--3.6 to 6.5 kg; source: KFM, Kleintierfarm Madoerin AG, Switzerland. The dogs had been dewormed and vaccinated for distemper, hepatitis, and parvovirus by the supplier.

B. STUDY DESIGN:

1. Animal Assignment: Following 17 days of acclimation, animals were assigned to the following test groups with a computerized randomization procedure:

Test Group	Nominal Daily Dosage (mg/kg/day)	Corrected Daily Dosage (mg/kg/day)[a]	Main Study (12 months) Males	Main Study (12 months) Females	Interim Sacrifice (6 months) Males	Interim Sacrifice (6 months) Females
1 Control	0	0	8	8	4	4
2 Low (LDT)	2.0	1.8	8	8	4	4
3 Mid (MDT)	5.0	4.5	8	8	4	4
4 High (HDT)	8.5	8.4[b]	8[c]	8[d]	3	3

[a] Calculated using mean values of the Nominal Daily Dosage (designated as Food Nominal Dosage Level by the study authors) and the results of analytically determined test material concentrations.

[b] High dosages adjusted from 10.8 to 8.4 mg/kg/day on study day 11.

[c] Animal No. 28 died on day 14.

[d] Animal No. 63 died on day 10.

Dogs were individually housed in environmentally controlled rooms with a 12-hour light/dark cycle.

2. Diet Preparation: The test compound was incorporated into standard laboratory diet (Kliba No. 355 dog maintenance diet) at either 2.0, 5.0, or 8.5 mg/kg/day bimonthly and stored at 22°C. Water was added in a 1:10 (v/w) ratio during pelleting of the diet preparation, after which the diet was air-dried for 48 hours and stored at room temperature. Control animals received a

similar diet without the test compound. Three hundred grams of the respective diets were offered to the dogs for 3 hours daily. The homogeneity and concentration of the test compound in the diet preparation were analyzed every 3 months. The stability of the test compound in the diet preparation was determined at 7-day intervals over a period of 21 days prior to study initiation and every 6 months thereafter. HOE 064706 (derived from HOE 039866 and supplied by the sponsor) was used as the analytical standard for gas chromatographic analyses.

Results: The test diet was found to be homogeneous within a range of ±10% over the study period, with the exception of the mid-dose mixture at week 50 which had a standard deviation of 19.7%. The mean concentrations of the low-, mid-, and high-dose diets were 92.8 ± 10.9 percent (range, 77.8-108.0 percent), 87.9 ± 11.1 percent (range, 76.0-94.9 percent), and 96.1 ± 10.6 percent (range, 84-108.3 percent), respectively. The test compound was found to be stable in the diet preparation for 21 days at room temperature. Stability data were reported for only the initial analysis conducted prior to study initiation.

3. Food and Water Consumption: Animals received 300 g of food (repelleted standard Kliba No. 335 dog maintenance diet) for 3 hours daily and water ad libitum.

4. Statistics: The following procedures were utilized in analyzing the numerical data. Body weights, food consumption, clinical pathology, and organ weights were analyzed using analysis of variance and Fisher's exact test. Control vs. treatment group proportions were compared using Student's T-test and William's test.

5. A quality assurance statement was signed and dated November 27, 1984.

C. METHODS AND RESULTS:

1. Observations: Animals were inspected twice daily for signs of mortality and morbidity.

Results: One high-dose male (animal No. 28) died on day 14 and one high-dose female (animal No. 63) was found dead on day 10. These deaths, considered to be caused by heart and circulatory system failure, were a result of rapid consumption of the 300-g daily food ration for approximately 2.5 days preceding death; intake of the test compound was reported to be significantly increased at this time when compared to that of the preceding 8-11 days. Two to 23 hours following this increased consumption of the test compound, symptoms of trismus (difficulty in opening mouth), and hyperactivity followed by somnolence and hypoactivity, tremor, tonic-clonic spasm, ataxia, stiff gait, opisthotonus (spasm), and lateral recumbency were reported for animal No. 28

on days 9-12 and 14 and for animal No. 63 on day 9. These same symptoms were reported in a second high-dose female (animal No. 61) on days 9 and 10; this animal survived by significantly decreasing food consumption. The two deaths were considered to be compound related; pathological examination reported myocardial necrosis and necrotizing aspiration pneumonia. The study authors felt that these findings indicated individual increased sensitivities of the dogs to HOE 039866 and a narrow dose-response relationship for the test compound. All other animals survived the dosing period.

Other observations, including hyperemic skin, alopecia, soft stool, and sporadic emesis, were not considered to be related to compound administration. Emesis and soft stool were reported to be found in control and dosed males and females. Individual data for clinical observations were not reported.

2. Body Weight: Dogs were weighed weekly.

   Results: The mean body weights of high-dose males were non-significantly reduced throughout the study when compared to concurrent controls (Table 1). Four mid-dose males (animal Nos. 18, 19, 20, and 21) displayed sporadic weight loss at week 11; however, this loss was transient and was not considered to be compound related. The body weights of mid- and high-dose females were slightly increased throughout the study when compared to concurrent controls.

3. Food Consumption and Compound Intake: Food consumption was determined daily. Compound intake was calculated from the consumption and body weight gain data.

   Results: The mean food consumption of dosed males was similar to that of concurrent controls with the exception of week 1 when the food consumption was slightly decreased in high-dose males. The mean food consumption of low-dose females was slightly decreased while that of mid- and high-dose females was slightly increased throughout the study when compared to concurrent controls.

4. Ophthalmological Examinations: Ophthalmological, auditory, and dental (teeth and mucous membranes) examinations were conducted prior to study initiation and at 3, 6, 9, and 12 months on all animals.

   Results: No compound-related effects were reported. Individual data for ophthalmological, auditory, or dental examinations were not reported.

5. Electrocardiogram (EKG): EKGs were conducted prior to study initiation, at 6 months on animals selected for the interim sacrifice, and at 12 months for all survivors; these tests were conducted 24 hours following the previous dosing period.

TABLE 1. Representative Results of Mean Body Weights (kg ± SD) of Dogs Fed HOE 039866 for 12 Months[a]

Dosage group (mg/kg/day)	Mean Body Weights at Week					
	Pretest	4	12	26	41	52
			Males			
0	6.57 ± 0.69	7.71 ± 0.72	8.92 ± 0.87[b]	10.23 ± 1.01	10.28 ± 1.39	10.12 ± 1.34
2	6.31 ± 0.53	7.38 ± 0.55	8.57 ± 0.56	9.72 ± 0.73	9.79 ± 0.90	10.04 ± 1.02
5	6.26 ± 1.09	7.22 ± 1.05	8.56 ± 1.09	9.69 ± 1.10	9.84 ± 0.93	9.99 ± 0.85
8.5	6.25 ± 1.04	6.98 ± 1.06	8.01 ± 1.28	8.91 ± 1.35	9.16 ± 1.86	9.42 ± 2.09
			Females			
0	4.92 ± 0.65	5.89 ± 0.91	6.98 ± 1.29	7.98 ± 1.61	7.91 ± 2.01	7.89 ± 2.37
2	4.83 ± 0.73	5.70 ± 0.86	6.68 ± 0.96	7.48 ± 1.08	7.23 ± 1.71	7.39 ± 1.95
5	5.08 ± 0.78	6.07 ± 0.88	7.25 ± 1.01	8.16 ± 1.03	8.51 ± 0.93	8.50 ± 0.92
8.5	5.00 ± 1.06	6.31 ± 0.77	7.76 ± 0.79	8.88 ± 0.93	9.47 ± 1.52	9.65 ± 1.13

[a]Based on eight animals/sex/dose from pretest to week 26 and four animals/sex/dose from weeks 27 to 52 with the exception of high-dose males and females with seven animals/sex from weeks 4 to 26 and four animals/sex from weeks 27 to 52.

[b]Standard deviation incorrectly reported by the study authors to be 0.37 kg.

Results: The heart rate of all dosed males and females was reported by the study authors to be decreased at 6 months; this decrease was dose related and significant ($p < 0.05$) at the high dose. The heart rate of dosed males and females was reported to be similar to concurrent controls at 12 months. All values were reported to be within the normal range of laboratory controls (normal values not reported). Individual EKG data were not reported. One high-dose male (animal No. 26) displayed a P pulmonale (increased amplitude of p-wave) at 12 months; the study authors were undecided regarding the cause of this finding. All other EKG findings were considered to be spontaneous and unrelated to dosing.

6. Hematology and Clinical Chemistry: Blood was collected from the jugular vein before treatment and at 1, 3, 6, and 12 months for hematology and clinical analysis from all animals. 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 corpuscular HGB concentration (MCHC)
X	Erythrocyte count (RBC)†	X	Mean corpuscular volume (MCV)
X	Platelet count†	X	Thromboplastin time (PT)
X	Reticulocyte count (RETIC)	X	Partial thromboplastin time (PTT)
X	Nucleated erythrocyte count	X	Thrombin time (TT)
X	Red cell morphology		
X	Bone marrow examination[a]		

Results: Hematology values for all animals were in the normal range; no compound-related findings were reported.

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[a] Conducted on control and high-dose males and females at the interim and terminal sacrifice; marrow was taken from the femur.

† Recommended by Subdivision F (October 1982) Guidelines.

b. Clinical Chemistry

	Electrolytes		Other
X	Calcium†		Albumin†
X	Chloride†	X	Blood creatinine†
	Magnesium†	X	Blood urea nitrogen†
X	Phosphorus†	X	Cholesterol†
X	Potassium†		Globulins†
X	Sodium†	X	Glucose
		X	Total bilirubin†
	Enzymes	X	Direct bilirubin†
X	Alkaline phosphatase (ALP)	X	Total protein†
	Cholinesterase		Triglycerides
X	Creatinine phosphokinase†	X	Uric acid
X	Lactic acid dehydrogenase		
X	Serum alanine aminotransferase (SGPT)†		
X	Serum aspartate aminotransferase (SGOT)†		
X	Gamma glutamyltransferase (GGT)		
X	Ornithine carbamyltransferase (OCT)		
X	Protein electrophoresis		
X	Bromosulfophthalein (BSP)[a]		
X	Phenolsulfonphthalein (PSP)[a]		

Results: Alkaline phosphatase (ALP) values were found to be slightly increased in males receiving 2.0 and 8.5 mg/kg/day at 1, 3, and 6 months; these values were significantly ($p < 0.01$) increased at 12 months when compared to concurrent controls (Table 2). ALP values were significantly increased ($p < 0.05$, $p < 0.01$) in males receiving 5.0 mg/kg/day throughout the study. However, these values were within the range of reference ALP values reported for laboratory controls and were not considered to be of toxicological significance. Other changes in clinical biochemistry parameters in males and females were sporadic and considered to be a result of random variation and not of toxicologic significance.

7. Urinalysis: Urine was collected from fasted animals prior to study initiation and at 1, 3, and 12 months. The checked (X) parameters were examined:

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

Results: There were no compound-related changes in urinary parameters.

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[a] Conducted on all animals at 6 and 12 months.

† Recommended by Subdivision F (October 1982) Guidelines.

TABLE 2. Mean Alkaline Phosphatase Values (ukat/L)[a] in Dogs Fed HOE 039866 for 12 Months[b]

Month	Dosage Level (mg/kg/day) 0	2.0	5.0	8.5
		Males		
Pretest	5.36	7.57	7.67	6.49
1	5.15	6.61	7.71*	6.78
3	3.81	4.99	5.78*	4.85
6	2.32	3.42	3.66*	3.25
12	1.40	2.84*	3.19**	2.69**
		Females		
Pretest	7.57	6.30	7.15	6.40
1	6.50	6.03	7.16	6.56
3	5.14	4.47	5.20	4.89
6	3.89	3.42	3.53	3.47
12	3.34	2.32	2.90	3.84

[a]Alkaline phosphatase is usually measured in IU/L (International Units/Liter); the units for alkaline phosphatase in this study were designated as ukat/L. The definition of this unit measurement and equivalency to IU were not provided.

[b]Standard deviations were not reported.

*Significantly different from control values at $p < 0.05$.

**Significantly different from control values at $p < 0.01$.

8. Sacrifice and Pathology: All animals that died or were sacrificed on schedule were subject to gross pathological examination, and the CHECKED (X) tissues were collected for histological examination. The (XX) organs from all animals were also weighed:

	Digestive system		Cardiovasc./Hemat.		Neurologic
X	Tongue	X	Aorta†	XX	Brain†
X	Salivary glands†	XX	Heart†	X	Peripheral nerve (sciatic nerve)†
X	Esophagus†	X	Bone marrow†	X	Spinal cord (3 levels)
X	Stomach†	X	Lymph nodes†	XX	Pituitary†
X	Duodenum†	XX	Spleen†	X	Eyes (optic nerve)†
X	Jejunum†	XX	Thymus†		
X	Ileum†				Glandular
X	Cecum†		Urogenital	XX	Adrenals†
X	Colon†	XX	Kidneys†		Lacrimal gland
X	Rectum†	X	Urinary bladder†	X	Mammary gland†
XX	Liver†	XX	Testes†	XX	Parathyroids†
X	Gallbladder†		Epididymides	XX	Thyroids†
X	Pancreas†	X	Prostate		
			Seminal vesicle		Other
	Respiratory	XX	Ovaries	X	Bone (sternum)†
X	Trachea†	X	Uterus†	X	Skeletal muscle†
XX	Lung†			X	Skin
				X	All gross lesions and masses

Results:

a. Organ Weights: The study authors reported that there were no changes in organ weights that were of toxicological significance (Table 3). Liver weights and liver-to-brain ratios were found to be nonsignificantly decreased in mid- and high-dose males at 6 months when compared to concurrent controls; however, liver weights of dosed males at 12 months were similar to control weights. Liver weights and liver-to-brain weight ratios of high-dose females were slightly increased at 12 months when compared to concurrent controls; these changes were not considered to be of toxicological significance.

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†Recommended by Subdivision F (October 1982) Guidelines.

TABLE 3. Mean Liver Weights, Liver-to-Body Weight Ratios, and Liver-to-Brain Weight Ratios in Dogs Fed HOE 039866 for 12 Months[a]

Dosage Group (mg/kg/day)	6 Months Absolute (g)	6 Months Relative Body (%)	6 Months Relative Brain (%)	12 Months Absolute (%)	12 Months Relative Body (%)	12 Months Relative Body (%)
			Males			
0	283.5 ± 22.6	2.67 ± 0.22	396.14 ± 46.49	243.9 ± 30.2	2.53 ± 0.17	320.99 ± 50.07
2	254.8 ± 36.4	2.58 ± 0.37	352.91 ± 64.78	249.0 ± 35.5	2.68 ± 0.21	334.15 ± 73.94
5	239.4 ± 27.3[b]	2.43 ± 0.21	338.50 ± 59.42	244.2 ± 27.2	2.63 ± 0.08	321.38 ± 44.61
8.5	238.4 ± 19.0	2.53 ± 0.22	328.11 ± 36.70	246.5 ± 61.2	2.76 ± 0.12	306.94 ± 72.63
			Females			
0	258.9 ± 55.1	3.23 ± 0.45	389.28 ± 89.14	219.9 ± 61.3	3.08 ± 0.54	327.81 ± 65.07
2	222.8 ± 50.9	3.01 ± 0.51	347.40 ± 68.12	199.3 ± 50.6	2.91 ± 0.20	293.39 ± 44.47
5	252.9 ± 21.0	3.26 ± 0.31	381.75 ± 42.32	227.3 ± 31.3	2.85 ± 0.10	313.39 ± 29.92
8.5	233.8 ± 18.8	2.98 ± 0.32	335.69 ± 3.65	290.6 ± 40.3	3.23 ± 0.39	393.45 ± 55.03

[a]Based on four dogs/sex/dose with the exception of high-dose males and females at 6 months with three dogs/sex/dose.

[b]Reevaluated by our reviewers using Duncan's multiple range test and found to be not significant; reported to be significant by study authors at $p < 0.05$.

b. Gross Pathology: Increased fluid in the pericardium and subendocardial hemorrhages of the right heart were found in the high-dose male that died during the study period. Numerous gray-green foci were found in the lungs of the high-dose female that died during the dosing period. Other gross pathological findings were considered to be incidental and not related to dosing administration.

c. Microscopic Pathology: Table 4 summarizes histologic findings for animals sacrificed at 6 months and study termination and those that died or were sacrificed moribund during the course of the study. Multifocal myocardial necroses were found in both high-dose animals that died during the study; severe necrosis was found in animal No. 28. In addition, severe necrotizing aspiration pneumonia, reported by the study authors to be a result of aspiration of diet material possibly ensuing from vomitus and somnolence, was found in animal No. 63. An acute gastric ulcer was also found in this animal. The study authors considered the myocardial necrosis to be compound related but were not conclusive regarding the cause of the gastric ulcer. The study authors reported that the findings in animals sacrificed at 6 and 12 months were spontaneous in origin and were normal age- and strain-related changes; these findings were seen in control and dosed animals.

D. STUDY AUTHORS' CONCLUSIONS:

Dietary administration of HOE 039866 to male and female beagle dogs for 12 months at concentrations of 2, 5, or 8.5 mg/kg/day produced no overt signs of toxicity in doses of 5 mg/kg/day or less. Two high-dose dogs died during the study period from heart and circulatory system failure. No indications of circulatory failure were seen in other animals. At sublethal dose levels, HOE 039866 was considered to induce circulatory failure.

E. REVIEWERS' DISCUSSION AND INTERPRETATION OF RESULTS:

The design and conduct of the study were acceptable; however, there were some deficiencies in data reporting. Clinical observations and eye examination results were not reported. Individual EKG data were not reported. Mean values for clinical pathology parameters were reported without standard deviations. A large variation existed between individual values for ALP measured in control and dosed animals throughout the study.

The mean absolute liver weight of males receiving 5 mg/kg/day was reported to be significantly ($p < 0.05$) decreased; these weights were reevaluated by our reviewer's using Duncan's multiple range test and found to be not significant.

TABLE 4. Representative Histological Findings in Dogs Fed HOE 039866 for 12 Months[a]

Organ/Finding	Dosage Group (mg/kg/day)							
	Males				Females			
	0	2	5	8.5	0	2	5	8.5
Heart	(8)[b]	(8)	(8)	(8)	(8)	(8)	(8)	(8)
Necrosis	0	0	0	1	0	0	0	1
Brain	(8)	(8)	(8)	(8)	(8)	(8)	(8)	(8)
Ventricular dilation	0	0	0	1	1	0	0	1
Lung	(8)	(8)	(8)	(8)	(8)	(8)	(8)	(8)
Perivascular cuffing	0	1	0	0	0	0	1	1
Granuloma	0	1	1	0	0	0	0	1
Liver	(8)	(8)	(8)	(8)	(8)	(8)	(8)	(8)
Round cell infiltration	1	2	2	3	0	1	3	2
Kupffer cell proliferation	1	0	2	3	1	1	2	0
Kidney	(8)	(8)	(8)	(8)	(8)	(8)	(8)	(8)
Lipid deposition	3	2	3	3	8	7	8	7
Spleen	(8)	(8)	(8)	(8)	(8)	(8)	(8)	(8)
Marginal hematoma	3	6	5	2	2	1	1	1
Stomach	(8)	(8)	(8)	(8)	(8)	(8)	(8)	(8)
Ulcer/erosion	0	0	0	0	0	0	0	

[a] Includes animals sacrificed at 6 months and at study termination and those that died or were sacrificed moribund during the course of the study.

[b] Numbers in parentheses are the numbers of tissues examined histologically.

The high-dose diet was adjusted from 10.8 to 8.4 mg/kg/day on study day 11; this preadjusted dosage exceeded the nominal high dose of 8.5 mg/kg/day. Three high-dose animals exhibited many compound-related symptoms, with deaths of two animals occurring on days 10 and 14. These deaths may have been attributed to excessive incorporation of HOE 039866 into the diet preparation.

We agree with the study authors that the two high-dose animal deaths were a result of compound-related circulatory system failure. Based on mortality, the systemic LOEL is 8.5 mg/kg/day.

Reviewed by: Whang Phang, Ph.D. 5/16/88
Section III, Tox. Branch (TS-769C)
Secondary reviewer: Marcia van Gemert, Ph.D. 5/17/88
Section III, Tox. Branch (TS-769C)

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DATA EVALUATION REPORT

STUDY TYPE: Mouse Oncogenicity Study

CHEMICAL: HOE 039866; Monoammonium [2-amino-4-(hydroxymethyl-phosphinyl)butanoate]; Ignite®

ACCESSION NUMBER: 403456-09 CASWELL NO.: 580I

EPA ID NO: 8340-EO/8340-EI PROJECT NO: 8-0146

SPONSOR: Hoechst Celanese Corp.

TESTING FACILITY: RCC, Research and Consulting Co. AG
P.O. Box CH-4452, Itingen, Switzerland

CITATION: Suter, P., Sachsse, K. et al. Two-year oncogenicity study with HOE 039866 technical in mice-dietary administration. Research and Consulting Co. AG, Switzerland. Project No.: 018527; April 29, 1986. Submitted by Hoechst Celanese Corp.

CONCLUSION

Groups of NMRI mice (50/sex/dose) were fed HOE 039866 at dietary concentrations of 20, 80, 160, or 320 ppm (160 ppm for males and 320 ppm for females) for 104 weeks. The means of actual compound intake are presented in Table 3. Additional groups of 10 mice/sex/dose were set aside for interim sacrifice at 52 weeks. The following compound-related effects were seen:

1. Dose-related increase in mortality rate in high dose (160 ppm) males.

2. Consistent and sometimes statistically significant decrease in body weight in high dose males (160 ppm) of the interim sacrifice animals.

3. Statistically significant increase in blood glucose levels in both high dose males and females at 52 weeks.

4. Decreases in albumin and total protein in high dose females at 52 weeks.

5. Decreases in GSH and slight increases in GSSG in whole blood of mid and high dose males and also in total GSH and GSSG level in whole blood of high dose males.

6. Decreases in absolute liver weight and in liver/body weight in all treated females.

7. Increase in incidences of cystic thyroid follicles and of chronic nephropathy in all treated males. It should be noted that these increases were not dose-related. The biological significance of these findings is not clear.

The tumor incidence of treated and control animals was comparable.

Based upon the decreased liver weights in all treated female mice, a NOEL for chronic toxicity of HOE 039866 could not be established. The study was well designed and conducted. This study is classified as Core Minimum according to the Agency's guidelilnes. However, with no NOEL, it is not useful for regulatory purposes.

A. MATERIALS:

1. Test compound was HOE 039866 technical with purity of 95.3%, and the chemical was a white crystalline powder.

2. Test animals: 4 week old NMRI mice which weighed 18-27 gm for males and 15-24 gm for females were used in this study. These animals were obtained from KFM, Kleomtoerfarm Madoerin AG, Switzerland.

B. STUDY DESIGN:

1. Animal assignment

Animals were acclimated for 10 days, and they were randomly assigned to the following test groups:

Test Group	Dose in diet (ppm)		Main Study† 104 weeks		Interim Sacr.* 52 weeks	
	Male	Females	male	female	male	female
1 Cont.	0	0	50	50	10	10
2 Low (LDT)	20	20	50	50	10	10
3 Mid (MDT)	80	80	50	50	10	10
4 High(HDT)	160	320	50	50	10	10

In the submission these groups were termed:
† oncogenicity study
* chronic toxicity study

The doses were selected based on the results of a 13-week feeding study in mice (RCC Project No: 018516). In this study, "a toxic effect at 320 ppm and more marked effect at 1280 ppm" were observed. Liver and kidneys were demonstrated to be the target organs, and males were more sensitive than females. Therefore, lower dose level was selected for group 4 males than that for group 4 females.

2. Diet preparation

The test chemical was mixed with diet to form pellets, and

water was added to aid pelleting. The treatment diet was prepared twice monthly and store at room temperature. Samples of the test diet were analyzed every 6 months for stability and homogeneity of the test chemical.

The results indicated that the mean concentrations of the test chemical in the diet were 102.6±16.9%, 98.7±12%, and 98.6±12.5%, of the targeted concentrations for groups 2, 3, and 4, respectively. The homogeneity varied from +20% to -18% of the mean concentrations of the analyzed samples. The test chemical was found to be stable for 21 days.

3. Animals received food and water ad libitum.

4. Statistical methods used for analyzing the results of this study are presented in Appendix 1.

5. Quality assurance statement was included in the report.

C. METHODS AND RESULTS:

1. Observations

Animals were inspected twice daily for signs of toxicity and mortality. In addition, each mouse was palpated weekly for tissue mass. Mortality is presented in Table 1.

For treated males in the main study, there was a dose-related increase in the number of animals that died prior to the scheduled sacrifice. In addition, the death rate in group 4 animals was significantly increased relative to that of the controls.

Table 1†

Mortality of Control and HOE 039866 Treated Mice

Group	Interim Sacrifice 52 weeks (10 mice/sex/dose)		Main Study 104 weeks (50 mice/sex/dose)		Total % of animals that died	
	Male	Female	Male	Female	Male	Female
1	0	1	26	42	43	72
2	0	1	29	39	48	67
3	0	0	31	35	52	58
4	1	0	38*	44	65	73

* Statistically significant ($p<0.05$; Fisher's exact test)

† Data excerpted from submission (EPA Accession No. 403456-09)

2. Body weight

Animals were weighed weekly during the first 3 months and subsequently twice monthly. The body weight data are presented in Appendix 2.

Main Study: In males, sporadic and significant decrease in body weight was observed in groups 3 and 4 animals. During the first 31 weeks of the study, the group 4 females showed frequent and statistically significant mean body weight depression, but subsequently the body weight of these females were comparable to that of the contols.

Interim Sacrifice: In males, reduced mean body weight (approximately 10%) was observed in group 4 animals through out the study (52 weeks). This reduction in mean body weight was statistically significant from weeks 3 to 33. In females, the mean body weights of the treated and control animals were comparable.

3. Food consumption and compound intake

Food consumption was recorded for a 7-day period, and the mean daily food consumption was calculated. There were isolated instances where statistically significant decreases or increases in food consumption were observed in both treated males and females. The over all average of the weekly means of food consumption were comparable between treated and control animals (Table 2).

TABLE 2*

Average of the Weekly Means of Food Consumption (gm/animal/day) for Control and HOE 039866 Treated Mice

	Interim Sacrifice		Main Study	
Group	Males	Females	Males	Females
1	5.9	6.8	6.3	6.6
2	6.6	7.0	6.4	7.1
3	7.0	6.8	6.2	7.0
4	6.0	6.8	6.2	6.4

* Data excerpted from submission (EPA Accession No. 403456-09)

Compound Intake: The means for daily compound intake were calculated and presented in Table 3.

TABLE 3†
Mean Daily Compound Intakes

	Males (M)	Females (F)
	mg/kg/day	mg/kg/day
Interim Sacrifice		
20 ppm	3.22	4.44
80 ppm	13.04	17.89
160(M)/320(F) ppm	25.04	69.15
Main Study		
20 ppm	2.83	4.23
80 ppm	10.82	16.19
160(M)/320(F) ppm	22.60	63.96

† Data excerpted from submission (EPA No. 403456-09)

4. Ophthalmological examinations were conducted on 10 mice/sex/dose at 6, 12, 18, and 24 months. No treatment-related effects were found.

Hearing test was performed on 10 mice/sex/dose using a tone generator (frequency, 10 KHz; volume, 80 dB; duration, 30 msec). The test was repeated 5 times with 2 sec pauses between each test. No hearing impairment was found in treated animals relative to the controls.

5. For hematology, blood samples were collected from 5 non-fasted mice/sex/dose at week 52 & 104. For differential white cell counts, samples were taken from the animals at terminal sacrifice. For clinical biochemistry, blood samples were collected from 5 fasted mice/sex/dose at weeks 52 and 104 weeks. The CHECKED (X) parameters were examined.

a. Hematology

X		X	
x	Hematocrit (HCT)*	x	Leukocyte differential count*
x	Hemoglobin (HGB)*	x	Mean corpuscular HGB (MCH)
x	Leukocyte count (WBC)*	x	Mean corpuscular HGB conc.(MCHC)
x	Erythrocyte count (RBC)*	x	Mean corpuscular volume (MCV)
x	Platelet count*	x	Reticulocyte count
	Blood Clotting Measurements	x	Red cell morphology
	(Thromboplastin time)	x	Heinz body
	(Clotting time)	x	methemoglobin
	(Prothrombin time)		

* Required for subchronic and chronic studies

For males, hematological parameters were comparable between treated and control animals. In group 3 females there were increases in RBC and decreases in MCV and MCH at 104 week. These changed were isolated, and a dose-related reponse was not observed.

b. Clinical Chemistry

X	Electrolytes:	X	Other:
	Calcium*	x	Albumin*
x	Chloride*	x	Blood creatinine*
	Magnesium*	x	Blood urea nitrogen*
	Phosphorous*	x	Cholesterol*
x	Potassium*		Globulins
x	Sodium*	x	Glucose*
	Enzymes	x	Total Bilirubin*
x	Alkaline phosphatase	x	Total Serum Protein*
	Cholinesterase#		Triglycerides
	Creatinine phosphokinase*°		Serum protein electrophoresis
x	Lactic acid dehydrogenase		
x	Serum alanine aminotransferase (also SGPT)*		
x	Serum aspartate aminotransferase (also SGOT)*		
x	gamma glutamyl transferase		
	glutamate dehydrogenase		

In blood and liver (Additional analyses)

x	reduced glutathione (GSH)
x	oxidized glutathione (GSSG)
x	Total glutathione (GSH + GSSG)

* Required for subchronic and chronic studies
\# Should be required for OP
° Not required for subchronic studies

In males rats, increased glucose levels were observed in group 4 animals at 52 week, but this change was not seen at 104 week (Table 4a). The level of GSH was decreased while that of GSSG was sligtly increased in whole blood of groups 3 and 4 males, and these changes were statistically significant (Table 4a). The total glutathione measurement was also significantly decreased in whole blood of group 4 males relative to that of the controls. Other changes were isolated and fluctuated in different dose groups.

In females rats, there were statistically significnat increases in glucose and SGOT levels and decreases in albumin and total protein in group 4 animals at 52 week (Table 4b). Cholesterol level was significantly decreased in all dosed females at 104 weeks. Additional changes in clinical biochemistry parameters were isolated and fluctuated among different dose groups.

7. Sacrifice and Pathology

All animals that died and that were sacrificed on schedule were subject to gross pathological examination and the

Table 4a

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CLINICAL BIOCHEMISTRY SUMMARY

MALES

	GLUCOSE mmol/l	UREA mmol/l	CREATININE umol/l	BILI. T. umol/l	CHOLEST.T. mmol/l	ASAT(GOT) ukat/l	ALAT(GPT) ukat/l	ALP ukat/l
52 WEEKS								
GROUP 1	8.65	7.27	45	4.0	3.08	1.35	1.27	2.26
GROUP 2	10.20	10.43	35	4.8	3.13	1.21	0.92	2.12
GROUP 3	9.78	7.22	43	5.0	3.16	1.64	1.19	1.99
GROUP 4	14.46 *	9.60	39	2.5	2.71	1.51	0.96	1.99

	G-GT ntat/l	SODIUM mmol/l	POTASSIUM mmol/l	CHLORIDE mmol/l	ALBUMIN g/l	PROTEIN T. g/l
AT 52 WEEKS						
GROUP 1	75.68	150.7	7.21	126.4	26.6	61.7
GROUP 2	101.69	154.0	9.29	122.4	26.5	59.6
GROUP 3	72.51	150.5	7.52	121.5	26.3	63.0
GROUP 4	82.10	147.8	7.50	125.3	27.4	59.0

MALES

	GLUCOSE mmol/l	UREA mmol/l	CREATININE umol/l	BILI. T. umol/l	CHOLEST.T. mmol/l	ASAT(GOT) ukat/l	ALAT(GPT) ukat/l	LDH ukat/l
104 WEEKS								
GROUP 1	7.33	9.34	37	4.0	3.38	2.69	3.64	14.20
GROUP 2	6.48	10.51	24	4.8	3.58	1.94	1.30	8.56
GROUP 3	7.01	8.17	29	4.0	4.24	2.47	3.43	10.95
GROUP 4	7.46	9.46	30	5.3	3.17	2.16	2.18	10.72

	ALP ukat/l	G-GT ntat/l	SODIUM mmol/l	POTASSIUM mmol/l	CHLORIDE mmol/l	ALBUMIN g/l	PROTEIN T g/l
AT 104 WEEKS							
GROUP 1	4.93	34.45	155.3	4.35	128.0	26.6	69.9
GROUP 2	3.14	26.67	158.3	4.57	126.3	27.3	68.0
GROUP 3	8.23	83.77	154.8	4.32	124.8	28.3	65.0
GROUP 4	4.03	60.01	156.8	4.80	127.4	25.9	58.5

	WHOLE BLOOD			TISSUE (LIVER)		
	GSH umol/ml	GSSG umol/ml	GSH+GSSG umol/ml	GSH umol/g	GSSG umol/g	GSH+GSSG umol/g
AT 104 WEEKS						
GROUP 1	0.72	0.18	0.90	2.25	1.67	4.07
GROUP 2	0.77	0.18	0.95	4.43 *	1.87	6.30
GROUP 3	0.57	0.27 *	0.84	4.25	1.79	6.04
GROUP 4	0.42 *	0.20	0.62 *	3.27	2.01	5.28

* : Dunnett-test based on pooled variance or Steel-test significant at 5% level

(DATA EXCERPTED FROM SUBMISSION)

TABLE 46

CLINICAL BIOCHEMISTRY SUMMARY
FEMALES

	GLUCOSE mmol/l	UREA mmol/l	CREATININE umol/l	BILI. T. umol/l	CHOLEST.T. mmol/l	ASAT(GOT) ukat/l	ALAT(GPT) ukat/l	ALP ukat/l
AT 52 WEEKS								
GROUP 1	8.66	7.57	35	5.9	3.20	1.36	1.11	3.45
GROUP 2	8.68	6.35	39	7.0	3.77	1.52	0.92	3.54
GROUP 3	6.87	8.04	44	4.3	3.91	1.41	1.47	2.77
GROUP 4	11.98 *	6.74	35	6.3	3.01	3.28 *	1.52	3.37

	G-GT nkat/l	SODIUM mmol/l	POTASSIUM mmol/l	CHLORIDE mmol/l	ALBUMIN g/l	PROTEIN T. g/l
AT 52 WEEKS						
GROUP 1	114.69	148.0	5.92	128.4	28.1	62.8
GROUP 2	94.60	148.1	5.09	128.4	29.7	64.2
GROUP 3	73.90	148.1	5.15	130.0	28.4	61.4
GROUP 4	69.68	148.2	6.02	128.0	23.6 *	55.3 *

	GLUCOSE mmol/l	UREA mmol/l	CREATININE umol/l	BILI. T. umol/l	CHOLEST.T. mmol/l	ASAT(GOT) ukat/l	ALAT(GPT) ukat/l	LDH ukat/l
AT 104 WEEKS								
GROUP 1	5.31	6.54	43	4.3	4.46	3.93	2.63	13.52
GROUP 2	4.98	8.02	26	4.6	2.61 *	1.48	1.28	7.12
GROUP 3	6.31	9.59	31	6.9	2.62 *	2.00	1.39	8.65
GROUP 4	5.60	7.22	23	5.3	2.48 *	2.88	1.51	15.99

	ALP ukat/l	G-GT nkat/l	SODIUM mmol/l	POTASSIUM mmol/l	CHLORIDE mmol/l	ALBUMIN g/l	PROTEIN T. g/l
AT 104 WEEKS							
GROUP 1	4.02	93.35	151.0	6.04	125.8	27.3	66.3
GROUP 2	4.68	45.01	151.2	4.78	127.4	28.8	64.8
GROUP 3	4.93	42.23	157.8 *	4.55 *	129.2	30.4	70.2
GROUP 4	6.08	61.68	153.2	4.74	131.3	28.0	64.5

	WHOLE BLOOD			TISSUE (LIVER)		
	GSH umol/ml	GSSG umol/ml	GSH+GSSG umol/ml	GSH umol/g	GSSG umol/g	GSH+GSSG umol/g
AT 104 WEEKS						
GROUP 1	---	---	---	3.05	1.88	5.19
GROUP 2	---	---	---	4.04	2.01	6.05
GROUP 3	---	---	---	3.93	1.70	5.63
GROUP 4	---	---	---	2.31	1.35	3.67

* Dunnett-test based on pooled variance or Steel-test significant at 5% level

DATA TAKEN FROM SUBMISSION

CHECKED (X) tissues were collected for histological examination. The (XX) organs in addition were weighed.

X		X		X	
	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)*#
x	.Stomach*	x	.Lymph nodes*	x	Pituitary*
x	.Duodenum*	xx	.Spleen*	x	Eyes (optic n.)*#
x	.Jejunum*	xx	.Thymus*		Glandular
x	.Ileum*		Urogenital	xx	.Adrenals*
x	.Cecum*	xx	.Kidneys*†		Lacrimal gland#
x	.Colon*	x	.Urinary bladder*	x	Mammary gland*#
	.Rectum*	xx	.Testes*†	x	.Parathyroids*††
xx	.Liver*†	x	Epididymides	xx	.Thyroids*††
x	Gall bladder*#	x	Prostate		Other
x	.Pancreas*	x	Seminal vesicle	x	Bone*#
	Respiratory	xx	Ovaries*†	x	Skeletal muscle*#
x	.Trachea*	x	.Uterus*	x	Skin*#
x	.Lung*			x	All gross lesions and masses*
	Nose°				
	Pharynx°				
	Larynx°				

* Required for subchronic and chronic studies
° Required for chronic inhalation
\# In subchronic studies, examined only if indicated by signs of toxicity or target organ involvement
† Organ weights required in subchronic and chronic studies
†† Organ weight required for non-rodent studies

a. Organ weight

After 52 weeks of treatment, absolute and relative spleen weights (spleen/body & spleen/brain) in dosed females were increased (Table 5). The increase in the high dose group was substantial, but it was not statistically significant because of large variability. A slight but statistically significant increase in absolute thyroid weight in mid dose females was also seen; this increase was not persistent. The organ weights of treated males were comparable to those of the controls.

After 104 weeks of treatment, significant decreases in absolute liver weight and in liver/body weight in all dosed females were found (Table 5). The reduced liver weight was not dose-related. After examining the individual animal data, it was found that although three control females had higher liver weights than other controls or treated animals, this variation could not account for reduced liver weights in these treated females (Table 5).

Table 5†

Absolute and Relative Organ Weights in Control and HOE 039866 Treated Female Mice

	0 ppm	20 ppm	80 ppm	320 ppm
After 52 Weeks				
Body wt	31.6±2.7	33.8±6.0	32.8±4.0	35.9±3.7
Brain wt	0.52±0.03	0.53±0.02	0.53±0.03	0.54±0.04
Spleen wt	0.106±0.021	0.134±0.061	0.0136±0.071	0.0218±0.212
Spleen/body wt	0.335±0.050	0.395±0.168	0.405±0.176	0.622±0.631
Spleen/brain wt	20.52±3.67	25.34±11.19	26.00±13.31	39.73±36.09
Thyroid wt	0.006±0.002	0.008±0.002	0.010±0.005*	0.009±0.003
After 104 Weeks				
Body wt	35.0±4.2	36.1±4.2	39.0±6.3	36.4±5.3
brain	0.54±0.05	0.51±0.02	0.51±0.02	0.50±0.02
Liver wt	2.96±1.01	2.01±0.45*	2.14±0.59*	1.94±0.54*
Liver/body wt	8.36±2.28	5.54±0.90**	5.46±1.14**	5.29±1.20**
Liver/brain wt	555.8±187.4	395.4±84.7*	417.8±105.1	389.06±108.5

* Dunnett-test based on pooled variance significant at 0.05
** Dunnett-test based on pooled variance significant at 0.01

† Data excerpted from submission (EPA Accession No. 403456-09)

TABLE 6

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PATHOLOGY REPORT
SUMMARY TABLES

PAGE : PAT 16
RCC-PROJECT: 018527

TEST ARTICLE : HOE 039866 TECHNICAL
TEST SYSTEM : MOUSE, 2 YEARS, FEEDING
SPONSOR : HOECHST AG, FRANKFURT/MAIN

EPS AG REF.: 86002 BSC
DATE : 09-JUN-86
EPS AG PATHDATA SYSTEM

NUMBER OF ANIMALS WITH NON-NEOPLASTIC LESIONS BY ORGAN/GROUP/SEX (KO)

	DOSE GROUP:	01		02		03		04	
	SEX:	M	F	M	F	M	F	M	F
KIDNEYS	NO.EXAM.:	50	50	50	50	50	50	50	50
- CORTICAL CYST(S)		28	7	25	6	30	6	21	6
- LYMPH.C.INFILTRATION		47	44	44	38	47	47	46	42
- TUBULAR ATROPHY		35	9	26	9	24	13	18	9
- TUBULAR DILATION		42	26	34	21	32	25	34	30
- GLOMERULOSCLEROSIS		2	12	10	19	5	12	6	13
- CHRONIC NEPHROPATHY		3	19	12+	27	13*	18	15*	18
- MINERALIZATION		3		4	1	10	3	3	1
- AMYLOIDOSIS			5	1	5	9	3		4
- INTERST. FIBROSIS				1		4	2		2
- PYELONEPHRITIS						2	2		
- GLOMERULONEPHRITIS							1		
- HYALINE DROPLETS					1	1			1
- TUBULAR VACUOLATION		1			1				
- PYELITIS				1		1			
- INTERST. NEPHRITIS			3		1				
- PELVIC DILATION			1		2		2		2
- INFARCT								1	
- ARTER-/PERIARTERITIS		1		5	4	7	1	5	
THYROID GLAND	NO.EXAM.:	50	49	50	47	50	49	50	50
- CYSTIC FOLLICLES		1	25	6	27	18*	34	17*	27
- FOLLICUL.HYPERPLASIA			1		1		1		2
- LYMPH.C.INFILTRATION		1	4		2	1	3	1	2
- CYST(S)		2							
- INFLAMMATION		2			3		1		3

DATA TAKEN From Submission

+ : Significant at $P < 0.05$ (Fisher's EXACT TEST)

* : Significant at $P < 0.01$ (Fisher's EXACT TEST)

Broad fluctuations in other organ weight values were observed, and the values of the treated animals were comparable to those of the controls.

b. Gross pathology

A summary of gross pathology findings was not reported. However, individual animal data did not indicate any consistent pattern for any effects observed in the treated animals.

c. Microscopic pathology

1) Non-neoplastic

A broad range of non-neoplastic lesions were observed in both treated and control animals. Most of these findings were comparable between treated and control animals except the incidences of cystic follicles of the thyroid and chronic nephropathy in treated male mice. Table 6 shows the non-neoplastic lesions in kidneys and thyroid of both control and treated mice. In treated males there were increased incidences of chronic nephropathy and cystic follicles of thyroid. The increase of chronic nephropathy was statistically significant in all treated males while that of cystic follicles of thyroid was significant only in mid and high dose animals. A clear dose-related effect was not observed in these animals.

2) Neoplastic Lesions

Various tumor incidences were observed in both treated and control animals, but these incidences were comparable between treated and control mice as shown in Appendix 3.

DISCUSSION

Groups of NMRI mice (50/sex/dose) were fed HOE 039866 at dietary concentrations of 20, 80, 160, or 320 ppm (160 ppm for males and 320 ppm for females) for 104 weeks and considered as the oncogenicity animals. Additional groups of 10 mice/sex/dose were fed similar concentrations of the test compound for 52 weeks, and these animals were considered as chronic study groups. The following observations were obtained with this experimental design:

1. Dose-related increase in mortality rate was observed in high dose (160 ppm) males of oncogenicity study.

2. Consistent and sometimes statistically significant decrease in body weight in high dose males (160 ppm) of the chronic study was found.

3. Statistically significant increase in blood glucose level in both high dose males and females of chronic study (52 weeks) was observed.

4. At week 104, Cholesterol levels of all treated females was significantly decreased relative to the controls. However, this decrease did not show a dose-related response and was considered as secondary to the effects of thyroid gland and liver. Individual animal data indicate that essentially all treated females, whose cholesterol levels were measured, had either cystic follicles of thyroid or infection of the liver with lymphocyte infiltration.

5. At 52 weeks, decreases in albumin and total protein were seen in high dose females.

6. At 104 weeks, decreased in GSH and increased GSSG in whole blood were found in mid and high dose males. Also, total GSH and GSSG level in whole blood was decreased in high dose males. These changes were compound-related.

7. Increased incidences of cystic follicles of thyroid and chronic nephropathy were observed in all treated males relative to that of the controls. Although these lesions did not show a clear dose-related response, the report did not present any explanation for the cause of this finding. It would be helpful if the registrant were to include the historical control data on the incidences of chronic nephropathy and cystic follicle of thyroid in NMRI mice of the performing laboratory.

8. Increased tumor incidence in treated male and female mice was not seen.

Based upon the increased incidences of cystic thyroid follicles and chronic nephropathy in all treated males and decreased liver weights in all treated female mice, a NOEL for chronic toxicity of HOE 039866 could not be established. This study is classified as Core Supplementary.

Appendix 1

(Information Excerpted from Submission)

RIN 5218-93 / Tox Review for Glufosinate Log # 293

Page_____ is not included in this copy.

Pages 193 through 214 are not included.

The material not included contains the following type of information:

____ Identity of product inert ingredients.

____ Identity of product impurities.

____ Description of the product manufacturing process.

____ Description of quality control procedures.

____ Identity of the source of product ingredients.

____ Sales or other commercial/financial information.

____ A draft product label.

____ The product confidential statement of formula.

____ Information about a pending registration action.

__✓__ FIFRA registration data.

____ The document is a duplicate of page(s) _____.

____ The document is not responsive to the request.

The information not included is generally considered confidential by product registrants. If you have any questions, please contact the individual who prepared the response to your request.

Reviewed by: Whang Phang, Ph.D. 6/23/88
Section III, Tox. Branch (TS-769C)
Secondary reviewer: Marcia van Gemert, Ph.D. 6/27/88
Section III, Tox. Branch (TS-769C)

DATA EVALUATION REPORT (DER)

006936

STUDY TYPE: Combined Chronic Toxicity and Oncogenicity Study-Rats

CHEMICAL: HOE 039866; Monoammonium [2-amino-4-(hydroxymethyl-phosphinyl)butanoate]; Ignite®

ACCESSION NUMBER: 403456-07 CASWELL NO.: 580I

EPA ID NO: 8340-EO/8340-EI

SPONSOR: Hoechst Celanese Corp.

TESTING FACILITY: RCC, Research and Consulting Co. AG
P.O. Box CH-4452, Itingen, Switzerland

CITATION: Suter, P., Sachsse, K. et al. Combined chronic toxicity / oncogenicity study in the rats - dietary administration. Research and Consulting Co. AG, Switzerland. Project No.: 018505; Report No.: A33811; Sept. 19, 1986. Submitted by Hoechst Celanese Corp.

CONCLUSIONS: Groups of Wistar rats were fed HOE 039866 at dietary concentrations of 0, 40, 140, and 500 ppm. The average compound intake calculated from food consumption and expressed as mg/kg are presented in the following table:

Average Compound Intake (mg/kg/day)

Group	Interim Sacrifice (52 & 104 wks)		Oncogenicity (130 wks)	
	Male	female	Male	female
1 (0 ppm)	0	0	0	0
2 (40 ppm)	2.1	2.5	1.9	2.4
3 (140 ppm)	7.6	8.9	6.8	8.2
4 (500 ppm)	26.7	31.5	24.4	28.7

Different groups received the test compound for various lengths of time; 10 rats/sex/dose were treated for 52 weeks; 20 rats/sex/dose, 104 weeks; 50 rats/sex/dose, 130 weeks. The following compound-related effects were observed:

1). Increased kidney glutamine synthetase activity in all treated females and in mid and high dose males.

2). Increased absolute and relative kidney weights in mid and high dose males and in all treated females.

3). Marginally increased incidence of "adrenal medullary tumor"

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in high dose males was observed. Although this increase was comparable to the incidence of the historical control data, additional information on specific description of the tumor types in adrenal medulla, and the range of the tumor incidence of the historical controls were lacking.

4). Based upon the data on survival rate, food consumption, and body weight, the highest dose for male rats did not approach the maximum tolerated dose (MTD).

Based upon increased kidney glutamine synthetase activity and absolute and relative kidney weights in all treated females, a NOEL could not be established. This study was well conducted; however, the classification of this study would be postponed until receipt and evaluation of the requested data.

A. MATERIALS:

1. Test compound: Technical grade HOE 039866, a white crystalline powder with purity of 95.3%.

2. Test animals: Four weeks old Wistar KFM-HAN rats which weighed 70-104 gm (males) and 50-73 gm (females) were obtained from KFM Kleintierfarm Madoerin AG, Switzerland.

B. STUDY DESIGN:

1. Animal assignment

According to the report, animals were assigned randomly to the following test groups:

Test Group	Concentration in diet (ppm)	INTERIM SACRIFICE* 52 weeks male	52 weeks female	104 weeks male	104 weeks female	ONCOGENICITY 130 weeks male	130 weeks female
1 (Cont.)	0	10	10	20	20	50	50
2 (Low)	40	10	10	20	20	50	50
3 (Mid)	140	10	10	20	20	50	50
4 (High)	500	10	10	20	20	50	50

* The Interim Sacrifice groups were considered as the Chronic Toxicity groups in the submitted report.

The dose levels were selected based upon the observations of 4-week and 13-week rat feeding studies. In the 4-week feeding study, "a tendency for increased kidney weights at 500 ppm and above" was

seen. In 13-week feeding study, slight increases in kidney weights and a decrease urine volume and an inhibition of liver glutamine synthetase at 500 ppm and above were observed.

2. Diet preparation

The test chemical was mixed with diet to form pellets, and water was added to aid pelleting. The treatment diet was prepared twice monthly and stored at room temperature. Samples of the test diet were analyzed at 3-month intervals for stability and homogeneity of the test chemical.

The results indicated that the mean concentrations of the test chemical in the diet were 95.8 ± 8.3; 97.4 ± 13.4; and 99.3 ± 9.5% of the nominal concentrations of 40, 140, and 500 ppm, respectively. The test chemical was found to be stable in the diet for least 21 days at room temperature.

3. Animals received food and water ad libitum.

4. Statistics - The following procedures were utilized in analyzing the numerical data:

A. For body weights, food consumption, organ weights and clinical laboratory data, univariate one-way analysis of variance was used to assess the significance of intergroup differences.

If the variance could be assumed to follow a normal distribution, the Dunnett-test based on a pooled variance estimate was applied for the comparison between the treated and control groups.

When the data could not be assumed to follow a normal distribution , the Steel-test (many-to-one rank test) was used.

B. Fisher's exact test was used to analyze spontaneous mortality data.

5. Quality assurance statment was signed and included in the report.

C. METHODS AND RESULTS:

1. Observations: Animals were inspected twice daily for signs of toxicity and mortality. Weekly clinical examination, which included palpation of tissue mass, was performed.

No compound-related toxic signs were observed in all treated animals.

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For oncogenicity animals, there was a dose-related increase in mortality of treated females relative to that of the controls (Table 1), and the increase in mid and high dose females was statistically significant compared to that of the controls. The cause of most deaths was reported to be incidental, and most of these incidental deaths were accompanied by pituitary adenomas in both treated and control females.

TABLE 1†
Mortality of Animals Treated with HOE 039866

Group	Number of Non-Schedule Deaths			
	Chronic Toxicity 30 rats/sex/group 52/104 weeks		Oncogenicity 50 rats/sex/group 130 weeks	
	Male	female	Male	female
1 [0 ppm]	6 (20)	4 (13)	25 (50)	15 (30)
2 [40 ppm]	4 (13)	5 (16)	27 (54)	23 (46)
3 [140 ppm]	5 (16)	3 (10)	27 (54)	27 (54)*
4 [500 ppm]	4 (13)	6 (20)	21 (42)	29 (58)*

( ): percentage
* $p < 0.05$ Pearson's Chi square test (conducted by this reviewer).
† Data excerpted from submitted report.

1. Body weight

Animals were weighed weekly for the first 3 months of the study and twice monthly thereafter. Several representative weekly means are presented in Table 2.

For the interim sacrifice animals, mean body weights of all treated males were increased at weeks 25 and 51; the increase in mid and high dose males was statistically significant. Mean body weights of low dose males and treated females were comparable to those of the controls at various measuring intervals.

For the oncogenicity animals, during the first year of the study low and high dose females showed increases in body weight relative to the controls. For males, only mid dose animals showed an increase in body weight at week 51, but the mean body weights of this group were greater than that of the controls prior to treatment. From week 112 to termination of the study, the body weights of all treated males were slightly decreased relative to that of the controls.

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TABLE 2†

Mean Body Weights of HOE 039866 Treated Animals (gm)

	0 ppm	40 ppm	140 ppm	500 ppm
A. Interim Sacrifice				
Male:				
25 weeks	441 ± 36	461 ± 51	470 ± 38*	473 ± 37**
51 weeks	253 ± 46	559 ± 80	563 ± 64*	564 ± 51*
104 weeks	588 ± 66	624 ± 89	628 ± 98	610 ± 69
Female:				
25 weeks	242 ± 20	243 ± 20	248 ± 21	242 ± 20
51 weeks	278 ± 26	281 ± 26	284 ± 27	284 ± 32
104 weeks	340 ± 44	344 ± 42	358 ± 45	346 ± 64
B. Oncogenicity				
Male:				
25 weeks	450 ± 39	463 ± 53	468 ± 41	462 ± 43
51 weeks	535 ± 47	551 ± 74	566 ± 62*	549 ± 58
105 weeks	613 ± 82	621 ± 90	619 ± 86	614 ± 74
130 weeks	567 ± 98	539 ± 68	518 ± 102	515 ± 80
Female:				
25 weeks	236 ± 23	246 ± 16*	242 ± 20	244 ± 18**
51 weeks	274 ± 34	285 ± 22	276 ± 29	294 ± 28**
105 weeks	346 ± 49	351 ± 44	335 ± 41	353 ± 49
130 weeks	338 ± 53	328 ± 43	319 ± 42	332 ± 47

Dunnet-Test based on pooled variance
* statistically significant at 0.05
** statistically significant at 0.01
† Data taken from submission

3. Food consumption and compound intake

Consumption was recorded, and mean daily diet consumption was calculated. Compound intake was calulated from food consumption data.

In interim sacrifice animals, food consumption for treated males was significantly increased at week 25/26 measuring interval relative to that of the controls (Table 3). A slight increase in food consumption was also observed in the the overall means for mid and high dose males relative to that of the controls. No difference in food consumption was observed between treated and control females.

In oncogenicity animals, increased food consumption was observed in both treated males and females relative to the controls at 25/26 week measuring interval (Table 3). However, this increase did not persist. The overall means for food consumption of all treated animals of both sexes were comparable to those of the controls.

The average compound intake by the experimental animals are presented in the following table:

Average Compound Intake (mg/kg/day)†

Group	Interim Sacrifice		Oncogenicity	
	Male	female	Male	female
1 (0 ppm)	0	0	0	0
2 (40 ppm)	2.1	2.5	1.9	2.4
3 (140 ppm)	7.6	8.9	6.8	8.2
4 (500 ppm)	26.7	31.5	24.4	28.7

† Data excerpted from the submitted report.

4. Ophthalmalogical examinations were performed on 10 rats/sex/dose at 12, 24, and 30 months of the study. No treatment-related effects were observed.

5. Blood was collected at 26, 52, 78, and 104 weeks for hematology and clinical analyses from 10 rats/sex/dose. Some of the methods applied are presented in Appendix I. The CHECKED (X) parameters were examined.

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TABLE 3†

Mean Food Consumption of HOE 039866 Treated Animals (gm)

	0 ppm	40 ppm	140 ppm	500 ppm
A. Interim Sacrifice				
Male:				
25/26 wks	22.0 ± 1.1	23.6 ± 0.6*	24.5 ± 0.9**	24.0 ± 1.2**
51/52 wks	21.8 ± 1.1	23.4 ± 1.1	23.9 ± 1.3*	22.8 ± 1.3
104 wks	21.6 ± 2.8	23.2 ± 1.0	24.0 ± 0.7	21.8 ± 3.0
Mean (overall)	21.9	22.7	23.8	23.1
Female:				
25/26 wks	15.2 ± 0.9	15.1 ± 1.2	15.5 ± 1.3	15.4 ± 0.4
51/52 wks	15.1 ± 0.5	15.4 ± 1.0	14.9 ± 1.2	15.4 ± 1.3
104 wks	16.7 ± 0.7	14.9 ± 3.1	17.8 ± 1.5	15.9 ± 2.6
Mean (overall)	15.6	15.1	15.8	15.4
B. Oncogenicity				
Male:				
25/26 wks	22.6 ± 1.3	23.8 ± 1.0*	23.8 ± 0.7*	23.4 ± 0.8
51/52 wks	22.8 ± 1.2	23.3 ± 1.2	23.5 ± 0.8	22.2 ± 1.0
104 wks	21.9 ± 2.6	23.2 ± 2.0	23.1 ± 1.7	23.0 ± 1.4
130/131 wks	20.1 ± 3.2	19.6 ± 4.0	18.6 ± 3.3	18.9 ± 2.8
Mean (overall)	22.4	22.8	23.0	22.8
Female:				
25/26 wks	14.3 ± 0.6	15.5 ± 0.8*	15.3 ± 0.7*	15.4 ± 0.8*
51/52 wks	14.4 ± 1.0	15.3 ± 0.6	14.7 ± 1.0	15.1 ± 1.1
104 wks	15.4 ± 1.0	16.6 ± 1.6	15.2 ± 1.2	14.8 ± 1.4
130/131 wks	15.6 ± 1.1	17.6 ± 2.5	14.8 ± 2.1	15.4 ± 1.6
Mean (overall)	15.2	16.0	15.3	15.7

Dunnet-Test based on pooled variance

* statistically significant at 0.05

** statistically significant at 0.01

† Data excerpted from submitted report

a. Hematology

X		X	
x	Hematocrit (HCT)*	x	Leukocyte differential count*
x	Hemoglobin (HGB)*	x	Mean corpuscular HGB (MCH)
x	Leukocyte count (WBC)*	x	Mean corpuscular HGB conc.(MCHC)
x	Erythrocyte count (RBC)*	x	Mean corpuscular volume (MCV)
x	Platelet count*	x	Reticulocyte count
x	Blood Clotting Measurements	x	Red cell morphology
x	(Thromboplastin time)	x	Heinz bodies
	(Clotting time)		
x	(Prothrombin time)		

* Required for subchronic and chronic studies

In high dose males and females, there were slight decreases in erythrocyte counts, hemoglobin concentration, and hematocrit and marginal increase in MCHC relative to those of the controls (Table 4). Most of these changes were observed at the after-52-weeks measurement period, and they were not considered to be treatment-related.

In high dose females, consistent and slight increase in mean corpuscular hemoglobin concentration (MCHC) was observe after 52 weeks of treatment (Table 4). However, this change was rather small and was not considered to be compound-related.

b. Clinical Chemistry: Some of the analytical methods are presented in Appendix 1.

X		X	
	Electrolytes:		Other:
x	Calcium*	x	Albumin*
x	Chloride*	x	Blood creatinine*
	Magnesium*	x	Blood urea nitrogen*
x	Phosphorous*	x	Cholesterol*
x	Potassium*		Globulins
x	Sodium*	x	Glucose*
	Enzymes	x	Total Bilirubin*
x	Alkaline phosphatase	x	Total Serum Protein*
x	Cholinesterase#		Triglycerides
x	Creatinine phosphokinase*°	x	Serum protein electrophoresis
x	Lactic acid dehydrogenase		
x	Serum alanine aminotransferase (also SGPT)*		
x	Serum aspartate aminotransferase (also SGOT)*		
x	gamma glutamyl transferase		
	glutamate denydrogenase		

TABLE 4

HEMATOLOGY SUMMARY
FEMALES

	RBC T/l	HB mmol/l	HCT l/l	MCV fl	MCH fmol	MCHC mmol/l	PLATELETS G/l	RETIC. %
AFTER 26 WEEKS								
GROUP 1	8.2	9.4	0.43	52.8	1.15	21.8	857	0.031
GROUP 2	8.2	9.4	0.43	52.3	1.16	22.1	899	0.029
GROUP 3	8.2	9.4	0.43	52.4	1.15	21.9	851	0.024 *
GROUP 4	7.9	9.1	0.42	53.2	1.15	21.8	869	0.026
AFTER 52 WEEKS								
GROUP 1	8.5	9.0	0.42	50.0	1.07	21.5	808	0.026
GROUP 2	8.2	8.9	0.40 *	49.0	1.08	22.1 *	818	0.021 *
GROUP 3	8.5	9.1	0.42	49.3	1.08	21.9	775	0.023
GROUP 4	7.9 *	8.7 *	0.39 *	49.0	1.10	22.4 *	799	0.028
AFTER 78 WEEKS								
GROUP 1	8.6	9.7	0.45	52.0	1.13	21.8	818	0.022
GROUP 2	8.8	9.9	0.45	50.9	1.12	22.1	855	0.021
GROUP 3	8.7	10.0	0.45	50.9	1.15	22.5 *	815	0.023
GROUP 4	8.5	9.9	0.44	52.0	1.17	22.4 *	827	0.023
AFTER 104 WEEKS								
GROUP 1	7.5	9.0	0.42	55.5	1.20	21.6	861	0.043
GROUP 2	7.6	9.1	0.41	54.2	1.20	22.1 *	899	0.046
GROUP 3	7.5	8.9	0.40	53.9	1.18	22.0	901	0.043
GROUP 4	7.5	9.2	0.41	54.6	1.22	22.3 *	850	0.040

MALES

	RBC T/l	HB mmol/l	HCT l/l	MCV fl	MCH fmol	MCHC mmol/l	PLATELETS G/l	RETIC. %
AFTER 26 WEEKS								
GROUP 1	8.9	9.7	0.44	49.0	1.09	22.3	884	0.023
GROUP 2	9.3	9.8	0.44	47.4	1.06	22.3	835	0.026
GROUP 3	9.2	9.7	0.44	47.4	1.05	22.2	815	0.027
GROUP 4	8.7	9.4	0.43	49.2	1.08	22.0	826	0.025
AFTER 52 WEEKS								
GROUP 1	9.4	9.3	0.42	45.0	0.99	22.0	901	0.021
GROUP 2	9.7	9.5	0.43	44.8	0.98	22.0	866	0.020
GROUP 3	9.6	9.3	0.43	44.4	0.97	21.9	850	0.024
GROUP 4	8.8	8.7 *	0.39 *	45.0	1.00	22.3	963	0.050
AFTER 78 WEEKS								
GROUP 1	9.3	10.2	0.45	48.2	1.10	22.9	926	0.020
GROUP 2	9.7	10.4	0.45	46.5	1.07	23.1	881	0.022
GROUP 3	9.8	10.3	0.45	45.6	1.05	23.0	958	0.022
GROUP 4	9.3	10.0	0.44	47.4	1.08	22.7	909	0.022
AFTER 104 WEEKS								
GROUP 1	8.4	9.3	0.42	50.6	1.11	21.9	936	0.034
GROUP 2	8.8	9.5	0.43	48.5	1.08	22.4	958	0.034
GROUP 3	8.6	9.3	0.42	48.3	1.08	22.3	982	0.036
GROUP 4	8.3	9.1	0.41	49.0	1.09	22.3	921	0.035

* Dunnett-test based on pooled variance or Steel-test significant at 5% level

Data abstracted from submission (EPA accession No. 403456-07)

Additional Clinical Biochemistry Analyses

x	Ornithine carbamyl transferase
x	Glutamine synthetase (liver, kidney, & brain; 10/sex/dose)
x	Amonia levels in liver, kidney, and brain (5/sex/dose)
x	Glutathione levels in liver and whole blood
x	Bromosulfophthalein (BSP) excretion test for liver function
x	Phenolsulfonphthalein (PSP) excretion test for kidney function

* Required for subchronic and chronic studies
‡ Should be required for OP
° Not required for subchronic studies

Reported results indicated no consistent changes in clinical chemistry parameters in treated males and females relative to the controls. However, the following changes were observed in the additional clinical chemistry analyses (Tables 5a & 5b):

a). In treated males and females, there was a dose-related increase in glutamine synthetase activity of the kidney (Tables 5a & 5b). The increase in females was statistically significant in all dose levels.

b). At the "after 52 weeks" examination period, there was an inhibition of glutamine synthetase activity of the liver in mid and high dose males and females (Tables 5a & 5b).

c). A decrease in brain glutamine synthetase activity was also observed in mid and high dose females at "after 52 weeks" and in low and high dose females at "after 104 weeks" examination intervals. The changes of glutamine synthetase activity in the brain of treated males were variable, and that of the controls and low dose males at "after 104 weeks" examination interval was not reported (Table 5a & 5b).

d). In treated male rats, the glutathione (GSH) level was decreased in whole blood of the high dose group. The oxidized glutathione (GSSG) levels were decreased in the liver of mid and high animals and increased in the whole blood of high dose group (Table 5a). In treated females, the GSH levels were significantly decreased in both liver and whole blood of mid and high dose animals (Table 5b).

e). The reported data on BSP and PSP excretion tests for liver and kidney function, respectively, were insufficient to determine the usefulness of these tests.

Table 5a

CLINICAL BIOCHEMISTRY
MALES

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SUMMARY

GLUTAMINE SYNTHETASE ACTIVITY

	LIVER ***	KIDNEY ***	BRAIN ***
AFTER 26 WEEKS			
GROUP 1	---	---	---
GROUP 2	---	---	---
GROUP 3	---	---	---
GROUP 4	---	---	---
AFTER 52 WEEKS			
GROUP 1	2.28	1.28	2.48
GROUP 2	2.07	1.41	2.48
GROUP 3	1.80 *	1.51 *	2.52
GROUP 4	1.71 *	1.72 *	2.34
AFTER 78 WEEKS			
GROUP 1	---	---	---
GROUP 2	---	---	---
GROUP 3	---	---	---
GROUP 4	---	---	---
AFTER 104 WEEKS			
GROUP 1	2.33	1.47	---
GROUP 2	2.39	2.03	---
GROUP 3	2.21	2.15 *	2.34
GROUP 4	2.35	2.35 *	2.19

	GLUTATHIONE (LIVER)			GLUTATHIONE (WHOLE BLOOD)		
	GSH umol/g	GSSG umol/g	GSH+GSSG umol/g	GSH umol/ml	GSSG umol/ml	GSH+GSSG umol/ml
AFTER 130 WEEKS						
GROUP 1	5.49	1.53	7.02	0.82	0.20	1.02
GROUP 2	5.37	1.43	6.80	0.76	0.19	0.95
GROUP 3	5.59	1.19 *	6.78	0.71	0.24	0.95
GROUP 4	6.02	0.98 *	6.99	0.54 *	0.32 *	0.85

* : Dunnett-test based on pooled variance or Steel-test significant at 5% level

***) Expressed in the tables as umol gamma-glutamyl-hydroxamate formed per ml reaction mixture / 20 min / 37 degrees centigrade

DATA ABSTRACTED From Submission (EPA Accession No. 403456-07)

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TABLE 56

CLINICAL BIOCHEMISTRY SUMMARY
FEMALES

	GLUTAMINE SYNTHETASE ACTIVITY		
	LIVER ***	KIDNEY ***	BRAIN ***
AFTER 26 WEEKS			
GROUP 1	---	---	---
GROUP 2	---	---	---
GROUP 3	---	---	---
GROUP 4	---	---	---
AFTER 52 WEEKS			
GROUP 1	2.85	0.79	2.35
GROUP 2	2.67	1.19 *	2.37
GROUP 3	2.38 *	1.27 *	2.19
GROUP 4	2.28 *	1.52 *	2.14
AFTER 78 WEEKS			
GROUP 1	---	---	---
GROUP 2	---	---	---
GROUP 3	---	---	---
GROUP 4	---	---	---
AFTER 104 WEEKS			
GROUP 1	2.92	1.19	2.53
GROUP 2	3.28	1.61 *	2.32 *
GROUP 3	3.56 *	2.01 *	2.44
GROUP 4	3.27	2.28 *	2.24 *

	GLUTATHIONE (LIVER)			GLUTATHIONE (WHOLE BLOOD)		
	GSH umol/g	GSSG umol/g	GSH+GSSG umol/g	GSH umol/ml	GSSG umol/ml	GSH+GSSG umol/ml
AFTER 130 WEEKS						
GROUP 1	4.98	1.32	6.30	0.98	0.25	1.23
GROUP 2	4.50	1.39	5.89	0.79	0.24	1.03
GROUP 3	3.10 *	1.26	4.36 *	0.66 *	0.25	0.90 *
GROUP 4	3.87 *	1.30	5.17 *	0.64 *	0.31	0.95

*** Expressed in the tables as umol gamma-glutamyl-hydroxamate formed per ml reaction mixture / 20 min / 37 degrees centigrade

* Dunnett-test based on pooled variance or Steel-test significant at 5% level

Data abstracted from submission (EPA Accession No. 403456-07)

f). There were no consistent changes in ammonia levels in plasma, liver, kidney, and brain of the treated animals relative to those of the controls.

6. Urinalysis°

Urine was collected from fasted animals at 26, 52, 78, and 104 weeks. Some of the analytical methods are presented in Appendix 1. 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*
x	Sediment (microscopic)*	x	Nitrate
x	Protein*	x	Urobilinogen

* Required for chronic studies
° Not required for subchronic studies

The results indicated that changes in urinary parameters were comparable between treated and control animals.

7. Sacrifice and Pathology -
All animals that died and that were sacrificed on schedule were subject to gross pathological examination and the CHECKED (X) tissues were collected for histological examination. The (XX) organs in addition were weighed.

X		X		X	
	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)*#
x	.Stomach*	x	.Lymph nodes*	xx	.Pituitary*
x	.Duodenum*	x	.Spleen*	x	Eyes (optic n.)*#
x	.Jejunum*	xx	.Thymus*		Glandular
x	.Ileum*		Urogenital	xx	.Adrenals*
x	.Cecum*	xx	.Kidneys*†		Lacrimal gland‡
x	.Colon*	x	.Urinary bladder*	x	Mammary gland*‡
x	.Rectum*	xx	.Testes*†	x	.Parathyroids*††
xx	.Liver*†	x	Epididymides	xx	.Thyroids*††
	Gall bladder*#	x	Prostate		Other
x	.Pancreas*	x	Seminal vesicle	x	Bone*‡
	Respiratory	xx	Ovaries*-	x	Skeletal muscle*#
x	.Trachea*	xx	.Uterus*	x	Skin*‡
xx	.Lung*			x	All gross lesions and masses*
	Nose°				
	Pharynx°				
	Larynx°				

* Required for subchronic and chronic studies
° Required for chronic inhalation
‡ In subchronic studies, examined only if indicated by signs of toxicity or target organ involvement
- Organ weights required in subchronic and chronic studies
†† Organ weight required for non-rodent studies

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a). Organ weights:

Organ weight data which showed any changes at different examination intervals are presented in Table 6.

At the "after 52 week" examination period, absolute heart and kidney weights were significantly increased in mid and high dose males (Table 6). The ratios of kidney to brain weight were also increased in these animals (Table 7).

At "after 130 week" examination period, statistically significant increase in absolute kidney weights of mid and high dose males was again apparent (Table 6). In addition, the ratios of kidney to body weight or to brain weight were significantly increased at "after 130 week" examination interval (Table 7).

In females, at "after 130 week" the absolute and relative kidney weights (kidney/body or kidney/brain) were increased in all treated groups (Tables 6 & 7). The increase was statistically significant in low and high dose females for absolute kidney weight; in all dose groups, for kidney/body weight; in high dose group, for kidney/brain weight.

b. Gross Pathology:

A summary table for gross pathology findings was not available in the report, but the individual animal data showed no apparent compound-related effects in the treated animals.

c. Microscopic pathology

1). Non-neoplastic:

For the oncogenicity rats, There was a marginal increase in incidence of interstitial fibrosis of the kidney in high dose males (24/49) relative to that of the controls (11/50), but this increase was not statistically significant. Other lesions were comparable between treated and controls animals.

2). Neoplastic:

In oncogenicity animals, a slight increase in benign adrenal medullary tumors was seen in mid and high dose males relative to the controls (Table 3). This increase did not show any statistical significance, and most of the tumors were found at week 130. However, when benign and malignant adrenal medullary tumors were combined, the increase was statistically significant for high dose males relative to the controls. In addition, the preliminary statistical analyses indicated a significant dose-related trend ($p=0.017$) for the combined adrenal medullary tumors.

TABLE 6†

Mean Organ Weights of HOE 039866 Treated Rats†

	0 ppm	40 ppm	140ppm	500 ppm
Males				
After 52 wks				
Heart	1.21±0.16	1.31±0.11	1.41±0.13*	1.40±0.18*
Liver	11.13±1.11	11.76±1.97	12.44±2.01	12.26±0.18
Kidney	2.23±0.24	2.47±0.25	2.70±0.25*	2.71±0.25*
Pituitary	0.01±0.002	0.013±0.004	0.009±0.002	0.012±0.003
After 104 wks				
Heart	1.37±0.21	1.38±0.17	1.41±0.14	1.45±0.17
Liver	16.36±2.83	16.20±2.16	16.90±2.30	15.77±2.17
Kidney	2.99±0.72	3.09±0.72	3.03±0.40	3.19±0.38
Pituitary	0.012±0.004	0.013±0.003	0.014±0.004	0.019±0.014
After 130 wks				
Heart	1.46±0.18	1.39±0.19	1.57±0.34	1.46±0.22
Liver	14.36±2.75	13.09±2.74	14.13±2.54	14.65±3.29
Kidney	3.00±0.37	3.08±0.58	3.64±1.00*	3.58±0.91*
Pituitary	0.023±0.028	0.035±0.050	0.045±0.066	0.031±0.043
Females				
Kidney				
After 52 wks	1.45±0.10	1.51±0.22	1.50±0.16	1.54±0.14
After 104 wks	1.80±0.21	1.84±0.13	1.92±0.23	1.95±0.31
After 130 wks	1.86±0.25	2.01±0.26*	1.96±0.22	2.09±0.22**

Dunett-Test based on pooled variance: * significant at 0.05
** significant at 0.01

† Data excerpted from the submission.

TABLE 7

Ratios of Kidney to Body Weight or to Brain Weight of HOE 039866 Treated Rats†

	0 ppm	40 ppm	140ppm	500 ppm
Males				
After 52 wks				
Kidney/Body wt	0.45±0.05	0.48±0.05	0.48±0.04	0.50±0.05
Kidney/Brain wt	104±11	112±13	123±13*	124±13*
After 104 wks				
Kidney/Body wt	0.52±0.15	0.51±0.10	0.50±0.09	0.53±0.06
Kidney/Brain wt	135±30	140±30	135±15	143±19
After 130 wks				
Kidney/Body wt	0.55±0.09	0.60±0.09	0.79±0.34**	0.75±0.28**
Kidney/Brain wt	131±17	137±26	163±47**	161±41**
Females				
After 130 wks				
Kidney/body wt	0.57±0.06	0.64±0.08**	0.67±0.10**	0.67±0.08**
Kidney/brain wt	92.2±13	99.56±16.1	97.5±12.3	102.2±10.1*

† Data excerpted from the submission.

Dunnett-Test based on pooled variance
* significance at 0.05
** significance at 0.01

The reported historical control data of the test laboratory indicated that the incidence of adrenal medullary tumor in Wistar male rats of less than 130 weeks old was 27/195 or approximately 14% (Appendix 2). Therefore, the adrenal medullary tumor incidence in HOE 039866 treated males was comparable to that of the historical controls.

Table 8††
Incidence of Adrenal Medullary Tumor in HOE 039866 Treated Wistar Male Rats

	0ppm	40ppm	140ppm	500ppm
Adrenal gland				
benign medullary tumor	1/50	1/50	3/50	5/50(10)
malignant medullary tumor	0/50	2/50	2/50	2/50(4)
Combined (malignant & benign)	1/50*	3/50	5/50	7/50(14)†

( ): percentage
* significant dose-related trend p = 0.017 with Cochran-Armitage test.
† significant at p = 0.0297 with Fisher's exact test.
†† Data excerpted from pages 886 & 917 of the report.

DISCUSSION

Groups of Wistar rats were fed HOE 039866 at dietary concentrations of 0, 40, 140, and 500 ppm. Different groups received the test compound for various lengths of time; 10 rats/sex/dose were treated for 52 weeks; 20 rats/sex/dose, 104 weeks; 50 rats/sex/dose, 130 weeks. The test animals which were treated for 52 and 104 weeks were considered as animals of the interim sacrifice study whereas those treated for 130 weeks were for oncogenicity study. The following observations were obtained with this experimental design:

1. The average compound intake per day for various groups were:

Average Compound Intake (mg/kg/day)†

Group	Interim Sacrifice		Oncogenicity	
	Male	female	Male	female
1 (0 ppm)	0	0	0	0
2 (40 ppm)	2.1	2.5	1.9	2.4
3 (140 ppm)	7.6	8.9	6.8	8.2
4 (500 ppm)	26.7	31.5	24.4	28.7

† Data excerpted from the submission.

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2. In interim sacrifice groups, all males showed increases in body weights during several examination intervals whereas the body weights of the treated females were essentially comparable to those of the controls.

   In oncogenicity groups, slight and variable increases in mean body weights were observed in treated males and females during the first year of the study. From week 112 to termination of the study, the mean body weights of all treated males were decreased relative to those of the controls, but the decrease was not statistically significant.

3. At the beginning of the study, treated males and females showed increased food intake, but this increase did not persist.

4. In males, marginal increase in MCHC and decreases in RBC, hemoglobin, and hematocrit were observed. Most of these changes were not persistent and were not considered as compound-related effects.

5. In mid and high dose males and all treated females consistent increases of kidney glutamine synthetase were observed. These increases were statistically significant and dose-related.

6. Increases in absolute and relative kidney weights (kidney/body & kidney/brain) were found in mid and high dose males, and these increases were statistically significant at various examination intervals. In females, increases in absolute and relative kidney weights were found in all dose groups at "after 130 week". Increases in absolute and relative kidney weights were also observed in all treated females of a 28-day feeding dose-range finding study in which 5 Wistar rats/sex/dose were administered HOE 039866 at dietary concentrations of 50, 500, 2500, and 5000 ppm.

7. At 130 week, a marginal increase in benign adrenal medullary tumor was observed in high dose males. When benign and malignant adrenal medullary tumors were combined, a statistically significant increase was found in high dose males relative to the controls. Although the increased incidence of combined adrenal medullary tumors in treated males showed a positive dose-related trend, the increased incidence of the adrenal medullary tumor was comparable to that of the historical controls of the testing laboratory.

   At present the oncogenic effects of HOE 039866 can not be properly determined because the diagnostic term "adrenal medullary tumor" may include ganglioneuroma, pheochromocytoma, and hemangioma of the adrenal medulla. It is recommended that the registrant reevaluate the appropriate pathology

slides and apply more specific descriptions for tumors found in the adrenal medulla. Similar descriptions for tumor types should also be applied for the historical control data specifially pheochromocytoma, malignant pheochromocytomas, and ganglioneuromas. In addition, control data of individual studies from which the combined historical control data were compiled should be clearly reported.

Based upon the statistically significant and dose-related increases in kidney glutamine synthetase activity in all treated females and increases in absolute and relative kidney weights in all treated females, a NOEL could not be established. The increase in glutamine synthetase activity in kidney demonstrated a pharmacological effect.

In addition, based upon the data on body weight, mortality rate, and food consumption, the highest dose for male rats had not approached the maximum tolerated dose (MTD).

This study has been well conducted; however, at present the classification of this study is postponed until receipt and evaluation of the requested information.

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Appendix I

(DATA TAKEN FROM Submission)
(EPA Accession No. 403456-07)

RIN 5218-93 / TOX Review for Glufosinate Log #293

Page_____ is not included in this copy.

Pages 235 through 248 are not included.

The material not included contains the following type of information:

____ Identity of product inert ingredients.

____ Identity of product impurities.

____ Description of the product manufacturing process.

____ Description of quality control procedures.

____ Identity of the source of product ingredients.

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____ A draft product label.

____ The product confidential statement of formula.

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____✓ FIFRA registration data.

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Review by: Whang Phang, Ph.D. 10/4/88 006936
Tox. Branch / HFAS
Secondary Review: Marcia van Gemert, Ph.D. 10/4/88
Tox. Branch / HFAS

ADDENDUM

(Hoe 039866; Ignite)
Combined chronic toxicity and oncogenicity study (rats)
(Hoechst Report No.: A33811; Sept. 19, 1986)

Introduction

In the combined chronic and oncogenicity study of Hoe 039866 on Wistar rats, marginally increased incidence of "adrenal medullary tumor" was observed in high dose males as shown in the following table.

Incidence of Adrenal Medullary Tumor in 130 Week Hoe 039866 Treated Wistar Male Rats

	0ppm	40ppm	140ppm	500ppm
Adrenal gland				
benign medullary tumor	1/50	1/50	3/50	5/50(10)
malignant medullary tumor	0/50	2/50	2/50	2/50(4)
Combined (malignant & benign)	1/50*	3/50	5/50	7/50(14)†

( ): percentage * significant dose-related trend p = 0.017 with Cochran-Armitage test.
† significant at p = 0.0297 with Fisher's exact test.

Data excerpted from pages 886 & 917 of the report (Hoechst Report No.: A33811)

The Toxicology Branch had requested additional information on the precise description of the "adrenal medullary tumor" and data on the tumor incidence of the historical controls of the performing laboratory.

Discussion:

In response, the registrant has submitted additional data. Relevant data are excerpted from the report and attachment. The term, "adrenal medullary tumor", refers to pheochomocytoma. The historical control data covers a period from 1985 to 1988. The incidence of benign pheochomocytoma in control Wistar rats ranged from 2% to 10%; that of malignant pheochromocytoma, from 0 to 16% (Tables 1 & 2). Based upon the newly submitted data, the marginally increased incidence of pheochromocytoma in male rats was within the range of the historical control incidence. In addition, pheochromocytomas have been commonly seen in male rats. Therefore, the oncogenic effects of the Hoe 039866 was not clearly demonstrated in this study.

This study was well designed and conducted. A NOEL could not be established because the lowest dose of Hoe 039866 (2.4 mg/kg) caused an increase in glutamine synthetase activity and kidney weights in female rats. Based upon the guidelines of the Agency, this study is classified as Core Minimum.

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TABLE 1+

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HISTORICAL CONTROL TUMOR INCIDENCE
WISTAR RAT

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ORGAN: ADRENAL GLANDS

PHEOCHROMOCYTOMA, BENIGN

(Synonym: medullary tumor, benign)

PROJECT	STUDY TYPE	REPORT	PATH.	GROUP	No. OF ORGANS EXAMINED		No. OF ORGANS WITH TUMORS		INCIDENCE IN %	
					M	F	M	F	M	F
005321	72 WEEK FEEDING	1985	JMA	01	50	44	2	-	4	0
				02	48	43	2	1	4	2
				03	3	8	-	-	-	-
				04	12	11	-	-	-	-
				05	46	43	-	1	0	2
006390	72 WEEK FEEDING	1985	RUD	01	50	50	2	1	4	2
				02	49	49	1	1	2	2
				03	14	11	1	-	-	-
				04	13	13	1	-	-	-
				05	50	48	-	-	0	0
017820	104 WEEK FEEDING	1986	HHW	01	49	50	2	1	4	2
				02	49	50	1	-	2	0
				03	50	50	2	1	4	2
				04	50	49	-	-	0	0
024300	104 WEEK FEEDING	1988	JMA	01	99	98	5	-	5	0
				02	50	50	3	1	6	2
				03	48	50	-	-	0	0
				04	48	49	-	1	0	2
027753	104 WEEK I. M.	1988	JMA	01	52	50	-	-	0	0
				02	13	18	-	-	-	-
				03	8	18	-	-	-	-
				04	54	53	1	1	2	2
008831	116 WEEK FEEDING	1986	PAG	01	49	50	2	-	4	0
				02	11	28	2	-	-	-
				03	13	21	1	1	-	-
				04	50	50	2	1	4	2
027472	120 WEEK FEEDING	1987	BSC	01	50	50	-	-	0	0
				02	49	49	1	-	2	0
				03	48	50	4	2	8	4
				04	50	50	1	1	2	2
004285	130 WEEK FEEDING	1985	BSC	01	49	50	3	-	6	0
				02	47	50	5	4	10	8
				03	50	49	4	2	8	4
				04	49	50	1	1	2	2
014387	130 WEEK FEEDING	1986	JAW	01	50	50	1	-	2	0
				02	49	49	1	-	2	0
				03	50	49	1	1	2	2
				04	49	49	-	1	0	2
				05	50	50	2	1	4	2
018505	130 WEEK FEEDING	1986	JAW	01	50	50	1	-	2	0
				02	50	50	1	3	2	6
				03	50	49	3	2	6	4
				04	50	48	5	1	10	2

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+ DATA TAKEN FROM THE REPORT (Supplement): EPA ACCESSION NO 407060-01

TABLE 2+

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HISTORICAL CONTROL TUMOR INCIDENCE
WISTAR RAT

ORGAN: ADRENAL GLANDS

PHEOCHROMOCYTOMA, MALIGNANT

(Synonym: medullary tumor, malignant)

PROJECT	STUDY TYPE	REPORT	PATH.		No. OF ORGANS EXAMINED M	No. OF ORGANS EXAMINED F	No. OF ORGANS WITH TUMORS M	No. OF ORGANS WITH TUMORS F	INCIDENCE IN % M	INCIDENCE IN % F
005321	72 WEEK FEEDING	1985	JMA	01	50	44	-	-	0	0
				02	48	43	-	-	0	0
				03	3	8	-	-	-	-
				04	12	11	-	-	-	-
				05	46	43	-	-	0	0
006390	72 WEEK FEEDING	1985	RUD	01	50	50	-	-	0	0
				02	49	49	-	-	0	0
				03	14	11	-	-	-	-
				04	13	13	-	-	-	-
				05	50	48	-	-	0	0
017820	104 WEEK FEEDING	1986	HHW	01	49	50	-	-	0	0
				02	49	50	-	-	0	0
				03	50	50	-	-	0	0
				04	50	49	-	-	0	0
024300	104 WEEK FEEDING	1988	JMA	01	99	98	3	1	3	1
				02	50	50	2	-	4	0
				03	48	50	-	-	0	0
				04	48	49	-	1	0	2
027753	104 WEEK I. M.	1988	JMA	01	52	50	2	-	4	0
				02	13	18	1	-	-	-
				03	8	18	-	2	-	-
				04	54	53	1	-	2	0
008831	116 WEEK FEEDING	1986	PAG	01	49	50	-	-	0	0
				02	11	28	-	-	-	-
				03	13	21	-	-	-	-
				04	50	50	-	-	0	0
027472	120 WEEK FEEDING	1987	BSC	01	50	50	-	2	0	4
				02	49	49	2	-	4	0
				03	48	50	-	-	0	0
				04	50	50	1	-	2	0
004285	130 WEEK FEEDING	1985	BSC	01	49	50	3	2	6	4
				02	47	50	2	1	4	2
				03	50	49	8	1	16	2
				04	49	50	1	1	2	2
014387	130 WEEK FEEDING	1986	JAW	01	50	50	-	-	0	0
				02	49	49	-	1	0	2
				03	50	49	2	1	4	2
				04	49	49	-	-	0	0
				05	50	50	-	-	0	0
018505	130 WEEK FEEDING	1986	JAW	01	50	50	-	-	0	0
				02	50	50	2	-	4	0
				03	50	49	1	2	2	4
				04	50	48	2	-	4	0

+ DATA TAKEN FROM THE REPORT (EPA ACCESSION No. 407064-0

Reviewed by: Whang Phang, Ph.D. 6/17/88
Section III, Tox. Branch (TS-769C)
Secondary reviewer: Marcia van Gemert, Ph.D. 6/17/88
Section III, Tox. Branch (TS-769C)

DATA EVALUATION REPORT

006936

STUDY TYPE: Teratology Study-Rats (Post-natal)

CHEMICAL: HOE 039866; Monoammonium [2-amino-4-(hydroxymethyl-phosphinyl)butanoate]; Ignite®

ACCESSION NUMBER: 403456-10 CASWELL NO.: 580I

EPA ID NO: 8340-EO/8340-EI EPA PROJECT NO: 8-0146

SPONSOR: Hoechst Celanese Corp.

TESTING FACILITY: Hoechst AG, 6230 Frankfurt am Main 80
Federal Republic of Germany

CITATION: Pensler, M. et al. HOE 039866-- Active ingredient Technical; Testing for embryotoxicity and effects on post-natal development in Wistar rats following oral administration. Hoechst AG: Study No. P2R0486; Report No. A33812. June 18, 1986; Submitted by Hoechst Celanese Corp., Somerville, NJ.

CONCLUSION:

Groups of 20 pregnant females rats were administered by gavage HOE 039866 at doses of 0.5, 2.24, and 10.0 mg/kg from day 7 to 16 of pregnancy. The dams were allow to deliver normally, and after birth the offspring were observed for 21 to 23 days. The results indicated that there were no significant differences in clinical observations, food consumption, body weights, implantations, resorption, and autopsy findings between treated and control dams.

In offspring, survival rates and body weight (at birth or sacrifice) were comparable between those from treated groups and those from controls. No increases in the incidence of soft tissue and skeletal anomalies were found in the offspring of the treated animals relative to those of the controls.

Based upon the findings presented in this report, No Observed Effect Level (NOEL) for maternal and developmental toxicity (post-natal) was 10 mg/kg/day (HDT). However, a dose greater than 10 mg/kg should have been tested in this study. Although the present study provided useful information, it used selective experimental conditions and did not meet the requirements for a teratology study. When the data of this study were considered with those of two previous studies (EPA Accession Nos.: 072965 & 073916), this study was classified as Minimum.

006936

METHODS AND MATERIALS:

1. Test compound: Technical grade HOE 039866; Serial No. 13143; purity, 96.9%.

2. Test animals: Sexually mature virgin Wistar rats of Hoe Wiskf (SPF71) strain. These animals were from Hoechst breeding colony, were approximately 10 week old, and weighed 194 ± 11.6 gm.

3. Study design: Prior to the administration of the chemical, the female rats in estrus were mated with fertile males. Those with sperm in the vaginal smear on the following morning were placed on the test. The day, when sperm was detected, counted as day 1 of pregnancy. From days 7 to 16 of pregnancy the females were administered the test agent daily by gavage. The pregnant females were randomly assigned to the following dose groups:

Group	Dose (mg/kg)	No. of Pregnant Females
1 (cont)	0 (distilled H_2O)	20
2 (Low)	0.50	20
3 (Mid)	2.24	20
4 (High)	10.00	20

The solution was prepared every 3 to 6 days by dissolving the test chemical in distilled water at concentrations of 0.100, 0.448 and 2.000 g/L. Each animal received an equal volume of 5 ml/kg body weight, and the body weight was based on current determination.

The general health and behavior of the test animals were evaluated daily. The body weights were measured weekly, at one day after the final treatment, and after delivery. Food consumption was determined during the pregnancy, but no further food consumption record was kept after delivery.

All pregnant females were allow to deliver normally and to rear their offspring for 21 days. All litters were weighed and examined after birth for external anomalies.

During the lactation period, the offspring were examined daily for viability, general behavior and any external anomalies. The body weights were measured on the day of delivery, on days 4 and 7 after delivery, and subsequently once weekly. The times of pinna separation, coat growth start, incisor eruption, and eyelid opening were noted.

The dams and offspring were sacrificed between days 21 and 23 post-partum; gross pathology was conducted on all animals. Heart, liver, kidneys, and spleen of all animals were weighed. The kidneys were also dissected along the longitudinal axis and examined for pelvic distension.

Offspring showing any signs of external anomalies were stained and examined for skeletal anomalies. The methods used for statistical evaluations were presented in Appendix 1.

RESULTS:

MATERNAL

1). Clinical observations: Essentially all animals survived to the end of the study except one dam each from 0.50 and 2.24 mg/kg groups. These two animals were sacrificed on day 25 because they had not littered by that day.

Table 1 shows a summary of clinical findings. There seemed to be a slight increase in the incidence of hair loss among high dose dams (5/20) relative to the controls (2/20). Also a small increase in the incidence of extended limbs was seen in high dose dams. Since these increases were so small, they were of doubtful toxicological significance.

2). Food consumption and body weight: The means of food consumption and of body weight for all treated and control animals were similar (Table 2).

3). Examinations at or after birth: Essentially all dams delivered between days 22 and 24 of pregnancy without complications. The parameters measured at birth and during weaning were similar among treated and control animals with the exception of one dam each in 0.50 and 2.24 mg/kg groups (Table 3). These two dams showed limited body weight gains relative to other dams and did not deliver on day 25 of pregnancy. They were sacrificed at that day. The 0.50 mg/kg dam showed empty implantation sites in the uterus, and the 2.24 mg/kg dam exhibited only 2 implantation sites and 1 dead fetus.

There was a slight increase in supernumerary implantation sites (resorptions) in the dams of 10.0 mg/kg groups (Table 3). However, this increase was not biologically significant under the current testing conditions because the increase was approximately 8%, and the implantation sites were examined 21 to 23 days post-partum.

The body weights of the offspring at birth were comparable between those of control and treated groups (Table 3).

3). Lactation period examinations: Survival rate and body weights of the offspring were comparable between offspring from the treated dams and those from the controls (Table 3). Significant increases in the incidence of external anomalies were not observed in any group of the test animals (Table 3).

4). Autopsy of dams and offsprings: The data showed that incidence of renal pelvic dilatation in either dams or offspring of the treated groups was comparable to that of the control animals (Table 4). No compound-related skeletal anomalies were reported.

TABLE 4†

Incidence of Hydronephrosis or Renal Pelvic Dilatation in Dams or Offspring, Respectively

	Control	0.50 mg/kg	2.24 mg/kg	10.00 mg/kg
Dams	1/20	3/20	2/20	2/20
Offspring	5/242	4/227	2/224	4/227

† Data excerpted from the submitted report (EPA Accession No. 403456-10).

5). Organ weights: There were no differences in organ weights between dams or offspring of the treatment groups and those of the controls (Tables 5 & 6).

DISCUSSION:

The results indicated that there were no significant differences in clinical observations, food consumption, body weights, implantations, resorptions, and autopsy findings between treated and control dams.

In offspring, survival rates and body weight (at birth or sacrifice) were comparable between those from treated groups and those from controls. No increases in the incidence of soft tissue and skeletal anomalies were found in the offspring of the treated animals relative to those of the controls. In contrast to the present study, a teratology study using higher doses (10, 50, & 250 mg/kg) of HOE 039866 and the same strain of rats

found an increase in the incidence of dilated renal pelvis and/or hydroureter in fetuses of all dose groups (Table 7)(EPA Accession No. 073916). However, an earlier teratology study using similar doses and strain of rats as the present study did not show an increase in the incidence of dilated renal pelvis (Table 8)(EPA Accession No. 072965). Much of the results of the present study appeared to be consistent with those of the earlier study.

Based upon the findings of the present study the No Observed Effect Level (NOEL) for maternal and developmental toxicity (post-natal) was 10 mg/kg/day (HDT). However, a dose greater than 10 mg/kg should have been tested in the present study. Although the present study provided useful information, it used selective experimental conditions and did not meet the requirements for a teratology study. When the data from this were considered with those from the previous two studies (EPA Accession Nos. 072965 & 073916), this study was classified as Minimum.

RIN 5218-93 / Tox Review for Glufosinate Log # 293

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Reviewed by: Whang Phang, Ph.D. 5/11/88 006936
Section III, Tox. Branch (TS-769C)
Secondary reviewer: Marcia van Gemert, Ph.D.
Section III, Tox. Branch (TS-769C) Management 5/13/88

DATA EVALUATION REPORT

STUDY TYPE: Teratology Study in Rabbits

CHEMICAL: HOE 039866; Monoammonium [2-amino-4-(hydroxymethyl-phosphinyl)butanoate]; Ignite®

ACCESSION NUMBER: 403456-11 CASWELL NO.: 580I

EPA ID NO: 8340-EO/8340-EI EPA PROJECT NO: 8-0146

SPONSOR: Hoechst Celanese Corp.

TESTING FACILITY: Hoechst AG, 6230 Frankfurt am Main 80
Federal Republic of Germany

CITATION: Baeder, C., Kramer, M., et al. HOE 039866-- Active ingredient Technical; Testing for embryotoxicity in Himalayan Rabbits Following Oral Administration. Study No.: G2K0402; Project No.: 84.0177, April 9, 1984; Submitted by Hoechst Celanese Corp., Somerville, NJ.

CONCLUSION Groups of pregnant Himalayan rabbits (15/sex/dose) were administered HOE 039866 (technical) by gavage at doses of 2.0, 6.3, and 20.0 mg/kg from gestation days 7 to 19. Fetuses were delivered on day 29 by caesarean section. The following findings were reported:

1. Increased incidences of premature delivery, abortion, or early resorption and decreased body weight and food consumption in 20 mg/kg dams.

2. Decreased food consumption in 6.3 mg/kg dams.

3. Increased number of dead fetus/litter in 20 mg/kg group.

4. Increased incidence of weak or absent ossification of some skeletal bones in fetuses of 6.3 and 20 mg/kg groups.

5. Increased kidney weights of 20 mg/kg dams.

This report has several deficiencies which include (1) discrepancies in food consumption and in body weight data between summary data and individual animal data, (2) incomplete food consumption data for individual 20 mg/kg dams, and (3) vague diagnoses of the skeletal examination of the fetuses. These deficiencies do not allow accurate assessment of the developmental toxicity of the test agent on rabbits; therefore, NOEL and LEL are not established under the present conditions. The study is classified as Supplementary.

A. MATERIALS:

1. Test compound: Technical grade HOE 039866; Serial No. 12027; purity, 95.3%.

2. Test animals: 6-7 months old Himalayan rabbits, which weighed 2547±203 gm, were obtained from Hoechst breeding colony.

B. STUDY DESIGN:

Pairing

The females rabbits were paired with known fertile males. When sperm was detected in the vaginal smear, they were paired again 6 hours later. The day when pairing occurred was counted as day 0 of gravidity. Pregnancy status was confirmed by the presence of implantation sites in the uterus.

Compound Administration

The dosages were freshly prepared every day by dissolving the test chemical in distilled water at concentrations of 0.40, 1.26, or 4.00 gm/L. An equal volume of 5 ml/kg body weights was administered to each animal between 8 and 11 AM. The dosage for each animal was adjusted according to the current body weight. The prepared solution was found to be stable for 5 days. The female rabbits were dosed by gavage from day 7 to day 19 after coitus.

The female rabbits were randomly assigned to the following dose groups:

Test Group	Dose mg/kg	Number of rabbits Day 7-19 of gravidity
1 Cont.	0	15
2 Low (LDT)	2.0	15
3 Mid (MDT)	6.3	15
4 High(HDT)	20.0	16

The above dosages were chosen based upon the results of a range-finding study with 2 pregnant Himalayan rabbits/dose. The doses were 10.0, 22.4, and 50.0 mg/kg. At 22.4 mg/kg, body weight loss was observed in both dams. During the second week, one of the dams refused to eat, and it showed 3 dead fetuses and a conceptus under resorption in the uterus. At 50.0 mg/kg, the dams showed marked weight loss and clinical signs such as head tilting and twitching, forelegs twitching, and extension spasms. One dam was sacrificed on day 14 of pregnancy, and the other died on day 15.

Clinical examinations

Behavior and general health conditions of the animals were observed daily. Food intake was measured. The animals were weighed weekly during the first 3 weeks and then once more after a 9-day period.

Examinations following caesarean section

On day 29, all fetuses were delivered by caesarean section, and the dams were sacrificed. Uterus and placenta were weighed and examined; corpora lutea on the ovaries were counted and examined.

The fetuses were examined for outward appearance and overt anomalies. Subsequently, the body weights of these fetuses were determined, and they were kept for 24 hours in an incubator. The dead fetuses were noted, and the crown-rump length of the fetuses was measured. The sex of the fetuses was determined at autopsy.

Approximately half of the fetuses of each litter and all fetuses which had been born before term, aborted, or found dead in the uterus were fixed in alcohol, dissected, eviscerated, and bleached in aqueous potassium hydroxide. The skeletons were stained with Alizarin Red S and examined for developmental anomalies.

The remaining fetuses and 3 prematurely born fetuses were fixed in Bouin's fluid and examined in body cross-sections under a stereomicroscope for organ anomalies.

After fetuses were delivered, the dams were dissected, and organs were grossly examined. Heart, liver, kidneys, and spleen were weighed.

Statistical evaluation

The statistical methods are presented in Appendix 1.

RESULTS

Maternal Toxicity

1). Clinical examinations

Clinical observations are presented in Table 1.

In 20.0 mg/kg group, one dam showed "extension spasm" for approximately 5 sec on day 16. Subsequently, this animal remained in high-legged position with head stretched and tilted. In the following morning this dam was lying in its stomach in a state of apathy, and it was sacrificed. Another 20.0 mg/kg dam aborted on the night of day 19 of pregnancy, and a third dam delivered prematurely in the night of day 24.

In addition, a 6.3 mg/kg dam died on day 29 while given

premature birth, and this animal was included under the premature deliveries.

The 2.0 mg/kg females showed no abnormal behavior or ill health conditions. They all survived until the end of the study.

Increased incidences of reduced water consumption and defecation were also observed in treated dams relative to the controls.

2). Food consumption

Food consumption was decreased in all treated dams relative to the controls during the treatment period (days 7 to 19) (Table 2a). The reduced food intake was statistically significant for 6.3 and 20.0 mg/kg dams during the measuring interval of day 14-20. However, when the treatment stopped (day 20), the mean food consumption of 6.3 and 20.0 mg/kg dams was comparable to or even slightly greater than that of the controls.

There were some discrepancies concerning the food consumption data. For example, in the individual animal data, values for food consumption were reported for 7 animals in 20.0 mg/kg group for days 20-29 of pregnancy, and the mean was reported to be derived from these values. However, the summary data as presented in Table 1a indicated that the mean was derived from 6 dams and was different from the mean derived from the individual animals. Similar discrepancies also existed for 6.3 mg/kg dams.

3). Body Weight

There was a significant decrease in body weight of 20.0 mg/kg dams at days 20 and 29 measuring intervals (Table 2b). It should be noted that the summary data for 20.0 mg/kg dams as presented in Table 2b could not be verified by the individual animal data, and a similar discrepancy as that seen in food consumption data also existed in body weight data for this group and 6.3 mg/kg group.

FINDINGS FOLLOWING CAESAREAN SECTION

1). Table 3 presents the summary of the results at caesaren section. Most of the parameters examined were comparable between treated and control dams except the following:

a). decreased number of dams with live fetus in 20.0 mg/kg group.

b. decreased body weight gain in all treated dams during pregnancy and that of 20.0 mg/kg group was more marked and statistically significant ($p < 0.05$).

c. Increased number of dead fetuses/litter was observed in all treated groups, and that of 20.0 mg/kg group was reported to be greater than the normal range of the historical controls of the performing laboratory.

2. Skeletal and soft tissue examinations

a. The summary of skeletal "anomalies", "variations", and "retardation" is presented in Tables 4a, 4b, and 4c. There were increases in the incidence of "weak or absent ossification one or more head bones" and "weak or absent ossification of pubis, calcancus, talus" in the fetuses of mid and high dose groups (Table 4c). However, these finding could not be verified by the individual animal data. For example, the individual animal data often described the finding as weak or absent ossification of individual skeletal bones. If all those findings were combined, the sum did not match summary data as reported.

b. There was no difference in soft tissue findings between treated and control groups (Table 5)

3. Organ weights of the dams

There was a slight and statistically significant increase in kidney weights of the high dose dams (Table 6). Liver weights of all treated dams were increased relative to those of the controls, but this increase was not statistically significant and reported to be within the normal range of the findings for the performing laboratory.

DISCUSSION

Groups of pregnant Himalayan rabbits (15/sex/dose) were administered HOE 039866 (technical) by gavage at doses of 2.0, 6.3, and 20.0 mg/kg from gestation days 7 to 19. Fetuses were delivered on day 29 by caesarean section. The following findings were reported:

1. In 20 mg/kg dams, increased incidences of premature delivery, abortion, or early resorption, and significantly decreased body weight and food consumption were observed.

2. Significant decrease in food consumption was also observed in 6.3 mg/kg dams.

3. In 20 mg/kg group, the number of dams with live fetus/litter

was decreased, and accordingly the number of dead fetuses/ litter was increased.

4. Increased incidence of weak or absent ossification of some skeletal bones were reported in fetuses of 6.3 and 20 mg/kg groups. However, the accurate magnitude of this increase was difficult to determine because the individual animal data on skeletal examination contained rather vague diagnoses.

5. Slight but statistically significant increase in kidney weights of 20 mg/kg dams.

This report has several deficiencies which include (1) discrepancies in food consumption and in body weight data between summary data and individual animal data, and (2) vague diagnoses of the skeletal examination of the fetuses. These deficiencies do not allow accurate assessment of the developmental toxicity of the test agent on rabbits; therefore, NOEL and LEL can not be established under the present conditions. The study is classified as Supplementary.

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006936

EPA: 68-02-4225
DYNAMAC No. 343F
May 18, 1988

DATA EVALUATION RECORD

IGNITE

Two-Generation Reproduction Study in Rats

STUDY IDENTIFICATION: Becker, H., Mueller, E., Frei, D., Vogel, W., Pappritz, G., Terrier, Ch., Sachsse, K., Ellehausen, H., and Chevalier, H. J. HOE 039866 technical (code: HOE 039866 OH ZC95 0001), multiple generation study in rats and preliminary study to the multiple generation study in rats. (Unpublished study Nos. A35589 and A33217 by RCC, Research and Consulting Co. AG, Intingen, Switzerland, for Hoechst Celanese Corp., Somerville, NJ; dated May 20, 1986.) Accession No. 403456-12.

APPROVED BY:

Robert J. Weir, Ph.D.
Acting Department Manager
Dynamac Corporation

Signature: Sharon Andrews for
Date: May 18, 1988

1. CHEMICAL: HOE 039866; Ignite; ammonium-(3-amino-3-carboxypropyl) methyl phosphinate.

006936

2. TEST MATERIAL: HOE 039866, code No. HOE 039866 OH ZC95 0001, was described as a hydroscopic white crystalline powder with a purity of 95.3%.

3. STUDY/ACTION TYPE: Two-generation reproduction study in rats.

4. STUDY IDENTIFICATION: Becker, H., Mueller, E., Frei, D., Vogel, W., Pappritz, G., Terrier, Ch., Sachsse, K., Ellehausen, H., and Chevalier, H. J. HOE 039866 technical (code: HOE 039866 OH ZC95 0001), multiple generation study in rats and preliminary study to the multiple generation study in rats. (Unpublished study Nos. A35589 and A33217 by RCC, Research and Consulting Co. AG, Intingen, Switzerland, for Hoechst Celanese Corp., Somerville, NJ; dated May 20, 1986.) Accession No. 403456-12.

5. REVIEWED BY:

Nancy E. McCarroll, B.S.
Principal Reviewer
Dynamac Corporation

Signature: Nancy E. McCarroll
Date: May 18, 1988

Guillermo Millicovsky, Ph.D.
Independent Reviewer
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Signature: G. Millicovsky
Date: MAY 18 1988

6. APPROVED BY:

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Date: May 18, 1988

Whang Phang, Ph.D.
EPA Reviewer

Signature: [illegible]
Date: 5/19/88

Marcia Van Gemert, Ph.D.
EPA Section Head

Signature: [illegible]
Date: 5/19/88

7. CONCLUSIONS:

A. The NOEL and LOEL for parental animals fed diets containing 0, 40, 120, and 360 ppm HOE 039866 are 40 and 120 ppm, respectively, based upon the absolute and relative kidney weight changes in the males and females of both generations fed 120 and 360 ppm.

The NOEL and LOEL for reproductive toxicity are 120 and 360 ppm HOE 039866, respectively, based on the significantly reduced number of pups per dam in all high-dose generations.

B. The study is classified Core Minimum.

Item 8--see footnote 1.

9. BACKGROUND:

A preliminary range-finding study was conducted with 0, 50, 500, 2500, and 5000 ppm HOE 039866 mixed with standard Kliba 343 rodent feed. Ten male and ten female Wistar/Han rats per group were administered the appropriate test diet for 3 weeks prior to mating and during mating, gestation, and lactation. Selected progeny were maintained on the test diets for 7 additional days. Results are summarized here and discussed in more detail in Section 12 (Reported Results).

No direct parental toxicity was seen at any dose. However, significant and dose-related preimplantation losses occurred at 2500 and 5000 ppm. At these levels, postimplantation loss was complete (100%); postimplantation loss at 500 ppm was 43%. Although the average litter size (6.4 pups/dam) for the 500-ppm dose group was significantly lower than the control group (12.7 pup/dam), no adverse effects on pup survival, development, or body weight were observed. The lower doses did not induce reproductive or developmental toxicity.

The investigators subsequently remated all males from the 0-, 500-, 2500-, and 5000-ppm dose groups with untreated females. In contrast to the implantation losses seen in the preliminary study, results for the supplementary test showed that pre- and postimplantation losses were markedly reduced when females were not treated.

Based on these findings, the study authors selected 40, 120, and 360 ppm as the dose levels for the multiple-generation study.

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[1]Only items appropriate to this DER have been included.

11. MATERIALS AND METHODS (PROTOCOLS):

A. Materials and Methods: (See Appendix A for details.)

1. Test Material: HOE 039866 technical (code No. HOE 039866 OH ZC95 0001), described as a hydroscopic white crystalline powder with a purity of 95.3%, was stored in the dark at -21°C. Diets containing 0 (control), 40, 120, and 360 ppm HOE 039866 were blended at least every 2 weeks with standard Kliba 343 rodent feed, pelleted, and stored at room temperature. Diet mixtures prepared for the preliminary study were analyzed for test material concentration, homogeneity, and stability. Feed batches were analyzed for test material concentration and homogeneity at selected intervals throughout the full study.

2. Animals and Experimental Design: Male and female Wistar/Han (Kfm: Wist, outbred, SPF) rats were obtained from Kleintierfarm, Madoerin AG, Fuellinsdorf, Switzerland. Following a 10-day acclimation period, 240 seven-week old animals (30 males and 30 females/group) were randomized, assigned to the test groups, and designated to be F_0 parental animals. All animals were maintained in an environment controlled for temperature (22 ± 2°C), relative humidity (55 ± 10%), and artificial light (12 hours/day). Tapwater and the appropriate test diet were provided ad libitum. Parental animals were individually housed except during mating.

After 80 days of treatment, females were paired with males from the same dose group for a maximum of 9 days to produce F_{1a} litters. Vaginal smears were examined daily for the presence of spermatozoa or a vaginal plug. The day either spermatozoa or a vaginal plug was noted was designated gestation day (GD) 0.

Approximately 10 days after weaning the F_{1a} litters, F_0 females were remated with different males of the same dose group for 8 days to produce F_{1b} litters.

Twenty-six male and 26 female F_{1b} weanlings per group were randomly selected as F_1 parents. After 101 days of treatment, F_1 adults were bred as described for the F_0 generations; sibling matings were avoided.

3. Observations and Measurements: Animals were examined twice daily for mortality and overt toxic signs. All males were weighed weekly except during mating. Females were weighed weekly prior to mating, on GD 0, 7, 14, and 21, and on days 1, 4, 7, 14, and 21 postpartum. Food consumption was determined weekly for each animal prior to mating; food consumption of the dams was recorded up to day 14 postpartum.

Item 10--see footnote 1.

Dams were observed twice daily near the end of gestation for signs of parturition; the day of parturition completion was designated day 0 postpartum. As soon as possible after parturition, pups were examined for abnormalities; litter sizes and sex ratios were determined and the numbers of live and dead pups were recorded.

Pups were weighed individually on days 1, 4, 7, 14, and 21 and observed daily for survival and behavioral abnormalities. Pups found dead were either necropsied or preserved in fixative.

On day 21 postpartum, one male and one female weanling per group per generation were randomly chosen from each litter. Selected organs from these weanlings were weighed, and tissues from the selected control and high-dose F_{2b} animals were examined microscopically. All other pups were subjected to gross necropsies and discarded.

Parental animals from both generations were necropsied and examined grossly; specific organs were weighed and tissues were preserved. Selected tissues from control and high-dose F_{1b} parents were examined histologically. The reproductive tracts of all parental males that failed to mate and all nonpregnant females were examined microscopically.

4. Statistical Methods: Body weight, food consumption, organ weight, and reproductive data were evaluated using one-way analysis of variance. Significant differences between groups were identified either by Dunnett's test or Steel's test. Overall values for spontaneous mortality were analyzed by Fisher's exact test.

3. Protocol: A protocol was not provided.

12. REPORTED RESULTS:

A. Preliminary Range-Finding Study:

No deaths occurred and no signs of toxicity were noted in parental animals fed 0, 50, 500, 2500, and 5000 ppm test diets. Throughout the 43-day treatment period, nonsignificant reductions in high-dose parental male body weights were observed; a similar trend occurred in high-dose females during premating and gestation. Due to the complete absence of fetuses in females of the 2500-and 5000-ppm groups, body weight determinations were discontinued for these animals. The lower-than-control body weight gains during the entire period of lactation in the 500-ppm group was caused by the marked reduction in litter sizes (6.4 pups/dam as compared to 12.7 pups/dam in the control group).

Males from the 500-, 2500-, and 5000-ppm dose groups were retained for further investigations. Organ weights were not

determined for the females of the 2500- and 5000-ppm dose groups. With the exception of a slight but nonsignificant increase in relative (to body) kidney weight of the 500-ppm females, organ weights for females of the 50- and 500-ppm dose group and low-dose males were comparable to the respective control weights. No macroscopic treatment-related lesions were seen in the sacrificed parental animals.

The test material had no effect on mating performance or pregnancy rate for groups exposed to ≤500 ppm, but the gestation length for the 500-ppm group (22.3 days) was approximately 1 day longer than for the control group. Although pregnancy was confirmed by the presence of implantation sites in the females of the 2500- and 5000-ppm dose groups, no fetuses were present.

Implantation data (summarized in Table 1) show that significant dose-related reductions in the number of implants occurred at 2500 and 5000 ppm. Percent postimplantation loss ranged from 43.4% at 500-ppm to 100% at both 2500 and 5000 ppm. Similarly, the mean number of total live pups per dam and male pups per dam in the 500-ppm group was significantly lower than those in the control group. Although 500, 2500, and 5000 ppm adversely affected implantation, the highest dose yielding litters (500 ppm) had no effect on pup survival, development, or body weight.

Terminal body weights as well as absolute liver and kidney weights for the 500-ppm male and female weanlings were significantly increased when compared to control group values; relative liver and kidney weights of females at both 50 and 500 ppm were also significantly increased. Gross necropsies, however, revealed no compound-related effects.

B. Supplemental Study:

In an attempt to ascertain if the compound effects on fertility were sex specific, the investigators conducted an additional experiment. For this study, the 0-, 500-, 2500-, and 5000-ppm F_0 males (10/group) were remated overnight with untreated mature females (10/group). Once mating was established, animals were individually housed, maintained on their respective diets (males on feed containing the test material; females on standard feed only) for 14 days. Males were necropsied and selected organs were weighed; the uterine contents of each female were examined and corpora lutea were counted.

TABLE 1. Summary of Implantation Data of Female Rats in the Preliminary Range-Finding and Supplementary Studies Conducted with HOE 039866

Preliminary Range-Finding Study[a]

Dose Level (ppm)	No. of Corpora Lutea	No. of Implants	No. of Live Pups[b]	Mean No. of Live Pups/Dam	Post-implantation Loss	Percent Postimplantation Loss
0	--	118	114	12.7	4	3.4
50	--	110	101	10.1	9	8.2
500	--	113	64	6.4*	49*	43.4*
2500	--	96*	--	--	--	100.0*
5000	--	80*	--	--	--	100.0*

Supplementary Study[c]

Dose Level (ppm)	Corpora Lutea	Implants	Percent Preimplantation Loss	Live Embryos	Mean percent Postimplantation Loss
0	11.8	11.3	4.2	10.9	3.5
500[d]	11.9	11.0	7.6	10.2	7.3
2500	12.2	11.7	4.1	9.8	16.2
5000	11.7	10.7	8.5	9.4	12.1

[a] Ten females per group.

[b] At parturition.

[c] The 10 treated males per group from the preliminary range-finding study were remated with 10 untreated females per group; one control group female was not pregnant.

[d] Individual raw data for the 500-ppm group was missing from the report.

* Significantly different from the control value (p value not reported).

Results from the necropsies performed on the males indicated that absolute and relative (to body) kidney weights for all three dose groups were increased; relative kidney weight increases for the 2500- and 5000-ppm males were significant. No treatment-related gross findings were noted at necropsy.

The breeding of treated males to untreated females had no adverse effects on mating performance or pregnancy rates.

As shown in Table 1, the percent pre- and postimplantation losses were generally higher among females mated to treated males as compared to the controls. However, since the percent losses were neither dose related nor significant, the authors considered these findings to be incidental to treatment. In contrast, the postimplantation losses in the preliminary study were significantly increased in the treated females when compared to the controls.

Based on the findings of the preliminary range-finding and supplemental studies, the authors concluded that,

> "The oral administration of the test article in the daily diet caused distinct effects on the reproduction of the females of groups 3 (500 ppm), 4 (2500 ppm) and 5 (5000 ppm) indicated by an increased postimplantation loss in group 3, and an increased preimplantation loss and a total postimplantation loss in groups 4 and 5. The no-observable-toxic-effect level was considered to be 50 ppm."

Dose levels established for the full study were 40, 120, and 360 ppm.

C. Multigeneration Study

Test Material Analysis: Chemical analysis of frozen diets yielded mean concentrations ranging from 102.1 to 108.7% of the nominal values. Homogeneity data ranged from +16 to -17% of the mean for top, middle and bottom samples.

Stability studies were conducted in the preliminary range-finding study on feed samples containing 50, 500, 2500, and 5000 ppm of the test material. Results for the 50- and 500-ppm doses, which were comparable to diet mixtures used in the current study, indicated that slight declines in test material concentrations occurred over the 21-day sampling interval (Table 2). Concentration decreases were noted for the 14- and 21-day analyses of 2500-ppm samples; however, the high-dose diet was consistently stable at room temperature for 21 days.

TABLE 2. Results of Stability Assays of HOE 039866 Technical in Rat Diets[a]

Nominal Concentration (ppm)	Percent Recovery at Room Temperature on Day			
	0	7	14	21
50	103.2	83.6	84.4	83.0
500	89.0	93.2	91.2	89.0
2500	90.7	90.4	69.6	75.6
5000	106.0	98.4	119.4	107.4

[a]These data were obtained from CBI Vol. 1, p. 257.

Parental Data: One mid-dose F_0 male was found dead on day 16 postcoitum and one low-dose F_0 female died 22 days after mating. Neither the cause(s) of death nor findings from necropsy (if performed) were reported; however, the study authors did not consider these deaths to be treatment related. Incidental findings of alopecia and skin lesions were seen in the control and dosed groups of both generations and sexes. As shown in Table 3, mean body weight for parental animals of both sexes and both generations were comparable to their respective controls throughout the course of the study. Similarly, body weight gains did not vary significantly among dosed and control groups. During lactation, a significantly lower amount of food was consumed by high-dose F_0 and F_1 dams rearing both litters; the difference in food consumption was, however, associated with reduced litter sizes.

Organ weight data (Table 4) indicated that both 120 and 360 ppm of the test material had a significant effect on F_0 male absolute and relative (either to body or brain) kidney, pituitary, and thymus weights. Similar significant findings were noted for kidney weights of the F_1 mid- and high-dose males and testicular weights in the high-dose group. Significant differences in organ weight parameters for F_0 females included low-dose pituitary and thymus weights (absolute and relative); mid-dose body-to-kidney weight; and high-dose ovary, thymus, and kidney (absolute and relative) weights.

In the F_1 females, significant findings were confined to the high-dose group and included absolute and relative liver, kidney, and thymus weights. Overall results suggest that, while significant differences were recorded for a variety of organs, only the findings for the increased kidney weights occurred with uniform frequency in the males and females of both generations. This effect, which was observed at both 120 and 360 ppm, is consistent with the kidney weight increases seen at higher doses in the preliminary studies.

Necropsy data for F_0 parents and mid- and low-dose F_1 parents were not presented; however, the pathology report stated that no treatment-related lesions were observed. Similarly, histological examinations of F_1 high-dose males and females indicated no adverse effects.

Reproductive/Developmental Data: Gross necropsies and microscopic examinations revealed no adverse effects on the reproductive organs of either sex. Gestation length were comparable for all groups of both generations. As shown in Table 5, the percentages of females mating and bearing live litters were generally comparable in all groups. The lower than expected number of live F_{1b} litters exposed to 40 ppm resulted from two dams cannibalizing their litters and one maternal death during parturition. Data presented for total and mean number of pups per dam showed that dams of both generations exposed to the high dose had reduced litter sizes; the finding was significant

TABLE 3. Mean Parental Body Weights (g) and Food Consumption of Rats Fed HOE 039866 Technical

	Dose Level (ppm)	Premating		Postmating (A litter)	Postmating (B litter)	Mean Food Consumption (g/animal/day)	
		Initial	Final[a]	Final[b]	Final[c]	Initial	Final
F_0 Males	0	151	397	449	471	22.4	22.6
	40	150	398	451	470	23.0	22.6
	120	147	396	450	476	22.1	23.1
	360	152	400	452	479	21.2	23.2
F_0 Females	0	128	230	277	296	16.1	61.8
	40	127	231	276	297	17.5	62.8
	120	126	227	270	297	17.2	60.9
	360	127	230	272	296	17.6	49.5*
F_1 Males	0	119	429	471	491	18.8	23.7
	40	121	427	468	495	18.3	23.9
	120	117	417	462	489	18.6	23.9
	360	120	431	479	504	18.5	24.4
F_1 Females	0	99	237	277	298	15.1	57.5
	40	108	244	278	298	15.1	57.4
	120	102	242	281	300	15.3	60.4
	360	107	249	284	308	15.4	50.0*

[a] F_0 male and female mean body weights on day 78; F_1 male and female mean body weights on day 100.

[b] F_0 male mean body weights on day 56; F_0 female mean body weights on day 21 postlactation; F_1 male weights on day 52; F_1 female mean body weights on day 21 postlactation.

[c] F_0 male mean body weights on day 42; F_0 female mean body weights on day 21 postlactation; F_1 male mean body weights on day 21 postcoitum; F_1 female mean body weights on day 21 postlactation.

*Significantly different from control value ($p < 0.05$).

TABLE 4. Mean Organ Weight Data of Rats Fed HOE 039866 Technical

	Dose Level (ppm)	Body Weight (g)	Absolute Organ Weight (g)				Organ:Body Weight Ratio (x100)			
			Kidneys	Pituitary	Thymus	Gonads	Kidneys	Pituitary	Thymus	Gonads
F_0 Males	0	467	2.3	0.011	0.37	3.7	0.49	0.002	0.08	0.79
	40	472	2.3	0.012	0.33	3.8	0.50	0.002	0.07	0.80
	120	480	2.5*	0.013*	0.27*	3.6	0.51[a]	0.003*[a]	0.06*[a]	0.74
	360	485	2.4*	0.013*	0.29*	3.8	0.50[a]	0.003*[a]	0.06*[a]	0.79
F_0 Females	0	285	1.8	0.017	0.19	0.11	0.63	0.006	0.07	0.038
	40	281	1.8	0.015*	0.24*	0.11	0.63	0.005*	0.09*	0.040
	120	279	1.8	0.017	0.23	0.11	0.66*	0.006	0.08	0.041
	360	283	1.9	0.016	0.26*	0.12*	0.66*[a]	0.006	0.09*[a]	0.042*[a]
F_1 Males	0	491	2.4	0.010	0.29	3.6	0.49	0.002	0.06	0.74
	40	495	2.4	0.011	0.29	3.7	0.50	0.002	0.06	0.75
	120	487	2.8*	0.011	0.31	3.7	0.57*[a]	0.002	0.07	0.76
	360	504	2.7*	0.011	0.30	4.0*	0.54*[a]	0.002	0.06	0.80*[a]
F_1 Females	0	296	1.9	0.015	0.15	0.10	0.64	0.005	0.05	0.032
	40	296	1.9	0.015	0.16	0.09	0.64	0.005	0.05	0.031
	120	296	1.9	0.016	0.18	0.09	0.64	0.005	0.06	0.032
	360	308	2.1*	0.015	0.22*	0.10	0.67[a]	0.005	0.07*[a]	0.033

*Significantly different from control values ($p < 0.05$).

[a]Organ-to-brain weight ratio was significantly different from the control value ($p < 0.05$).

TABLE 5. Summary of Reproductive Data of Rats Fed HOE 039866 Technical

Litter Generation	Dose Level (ppm)	No. Paired	Percent Mated[a]	Pregnant		Live Litters		Percent Viability[b]	Mean No. Viable Pups/ Litter
				No.	Percent	No.	Percent		
F_{1a}	0	30	100	27	90	27	100	99.7	11.2
	40	30	100	28	93	28	100	99.7	11.6
	120	30	100	29	97	29	100	99.7	10.6
	360	30	100	29	97	29	100	98.4	8.8*
F_{1b}	0	30	100	29	97	29	100	100	11.7
	40	30	100	28	93	25	89.3[c]	100	11.6
	120	30	100	29	97	29	100	100	11.3
	360	30	100	30	100	30	100	100	7.4*
F_{2a}	0	26	100	25	96	24	96	100	10.8
	40	26	100	25	96	25	100	99.6	10.9
	120	26	100	26	100	25	96	100	10.7
	360	26	100	25	96	24	96	100	9.6
F_{2b}	0	26	100	25	96	25	100	100	11.2
	40	26	100	25	96	25	100	99.7	11.8
	120	26	100	25	96	24	96	100	11.9
	360	26	100	26	100	26	100	98.6	8.2*

[a] Percent mated of females that were paired.

[b] Percent viable of total pups delivered.

[c] Two dams cannibalized their litters and one dam died during parturition.

* Significantly different from the control value ($p < 0.05$).

for F_{1a}, F_{1b}, and F_{2b} litter intervals. Based on these observations and the similar findings from the preliminary study, the authors concluded that the decrease in the total number of pups was indicative of test material interference with implantation due to toxic effects on early embryonic development.

The test material had no effect on the survival or weights of pups from any litter interval or generation (Table 6).

In general, terminal body weights of high-dose weanlings were either comparable to or higher than the respective control group (Table 7). The increases in terminal body weights were significant for both sexes of the F_{1b} generation and F_{2b} females. For these groups, significant increases in absolute and relative (to brain) kidney weights were noted. Other organs showing significant absolute and relative weights included adrenals and thymus for F_{1b} males, thymus for F_{1b} females, and heart, adrenals, and pituitary, for F_{2b} females.

The report stated that no treatment-related lesions were observed at necropsy. They, however, noted that one F_{2b} control female and two high-dose F_{2b} females had very small pituitary glands. Although the report indicated that histological evaluation of high-dose and control F_{2b} pups revealed no treatment-related findings, the incidence of lung atelectasis in high-dose F_{2b} pups was higher than that of the controls (42% combined for F_{2b} males and females vs. 32% combined for controls). Similarly, 31% of F_{2b} males and females had erythrophagocytosis of the mandibular lymph node as compared to a combined 14% for the controls.

13. STUDY AUTHORS' CONCLUSIONS/QUALITY ASSURANCE MEASURES:

A. The study authors concluded that parental animals and progeny of all generations tolerated test article concentration levels up to and including 360 ppm in the diet without any toxic or teratogenic effects.

The "no-observable-effect level" is considered to be 120 ppm, due to the reduced litter sizes (number of pups per dam) noted in all group 4 litters of both generations. These effects were caused by pre- and postimplantation losses in the parent females as proven by the results of the preliminary study (RCC Project 018472) to this multiple-generation reproduction study.

B. A quality assurance statement was signed and dated August 11, 1987.

TABLE 6. Mean Litter Data of Rats Fed HOE 039866 Technical: Summary of Pup Survival and Body Weights

Litter Generation	Dose Level (ppm)	No. of Live Pups on Day 1[a]	No. of Live Pups on Day 21	Percent Survival on Day 21	Pup Weights (g) on Day 1[a]	Pup Weights (g) on Day 4	Pup Weights (g) on Day 21
F_{1a}	0	301	297	99	5.8	8.4	37.1
	40	326	319	98	6.0	8.6	36.3
	120	307	302	98	6.1	8.6	35.7
	360	254	251	99	6.3	9.1	37.8
F_{1b}	0	339	328	97	5.8	8.7	40.2
	40	289	283	98	6.0	9.0	41.7
	120	327	321	98	6.1	8.9	40.7
	360	222	220	99	6.5	9.8	45.2
F_{2a}	0	259	250	96	5.8	8.5	37.1
	40	272	264	97	5.8	8.5	35.0
	120	268	265	99	6.1	9.0	38.6
	360	231	212	92	5.9	8.7	36.9
F_{2b}	0	280	275	98	6.1	8.7	40.5
	40	295	289	98	5.9	8.5	38.5
	120	285	279	98	6.1	8.9	40.7
	360	214	211	99	6.3	9.4	43.6

[a]Recorded within 16 hours of parturition.

TABLE 7. Mean Organ Weight Data of Weanlings Fed HOE 039866 Technical

	Dose Level (ppm)	Body Weight (g)	Absolute Organ Weight (g)		Organ:Body Weight Ratio (x100)	
			Kidney	Gonads	Kidney	Gonads
F_{1a} Males	0	38	0.4	0.21	1.06	0.6
	40	37	0.4	0.20	1.04	0.5
	120	37	0.4	0.20	1.05	0.6
	360	39	0.4	0.21	1.06	0.5
F_{1a} Females	0	37	0.4	0.02	1.08	0.05
	40	36	0.4	0.02	1.07	0.06
	120	36	0.4	0.02	1.06	0.06
	360	38	0.4	0.02	1.08	0.05
F_{1b} Males	0	42	0.4	0.22	1.00	0.5
	40	43	0.4	0.21	1.01	0.5
	120	42	0.4	0.21	1.01	0.5
	360	47*	0.5*	0.24	1.01[a]	0.5
F_{1b} Females	0	41	0.4	0.02	1.03	0.05
	40	42	0.4	0.02	1.04	0.04
	120	40	0.4	0.02	1.05	0.04
	360	45*	0.5*	0.02	1.07[a]	0.04
F_{2a} Males	0	39	0.4	0.21	1.01	0.6
	40	37	0.4	0.19	1.03	0.5
	120	40	0.4	0.21	1.03	0.5
	360	38	0.4	0.19	1.02	0.6
F_{2a} Females	0	38	0.4	0.02	1.04	0.05
	40	36	0.4	0.02	1.06	0.05
	120	39	0.4	0.01	1.08	0.04
	360	36	0.4	0.01	1.03	0.04
F_{2b} Males	0	42	0.4	0.22	1.03	0.5
	40	40	0.4	0.20	1.01	0.5
	120	41	0.4	0.22	1.04	0.5
	360	45	0.5	0.23	1.01	0.5
F_{2b} Females	0	40	0.4	0.02	1.04	0.05
	40	39	0.4	0.02	1.04	0.05
	120	41	0.4	0.02	1.08	0.05
	360	44*	0.5*	0.02	1.05[a]	0.04

*Significantly different from control values ($p < 0.05$).

[a]Organ-to-brain weight ratio was significantly different from the control value ($p < 0.05$).

14. REVIEWERS' DISCUSSION AND INTERPRETATION OF STUDY RESULTS:

A. Test Material Analyses: The results of chemical analyses indicated that the test diets were accurately prepared and homogeneously distributed in the diet mixtures. Data from the preliminary range-finding study demonstrated that, at concentrations comparable to those used in the current study, diet storage procedures were adequate to ensure test material stability under experimental conditions.

B. Parental Data: We assess that the test material had no effect on the body weight or the food consumption of parental animals of any generation.

At 120 and 360 ppm, significant increases in kidney weights were seen in both sexes and both generations. These findings were consistent with the results of the preliminary study, which showed similar effects at higher doses. We conclude, therefore, that the increased kidney weights were compound related, and indicate parental toxicity at 120 and 360 ppm.

Reproduction/Developmental Data: The test material had no effect on mating or pregnancy rates. We consider the significant reduction in the number for pups of all generations to be indicative of developmental toxicity at 360 ppm. Surviving pups and weight of the survivors were unaffected by treatment. We further assess that the combined results of the preliminary and complete studies provide evidence for reproductive toxicity in support of the authors' overall conclusion that HOE 039866, at doses greater than or equal to 360 ppm, adversely affects early embryonic development. The significant increases in kidney weights of the high-dose generations is of interest because of the consistency of these findings in both parents and offspring of both sexes and generations. Neither reproductive nor developmental toxicity was seen in the mid- or low-dose groups.

C. We assess that the NOEL and LOEL for parental toxicity are 40 and 120 ppm, respectively, based upon the increased kidney weights in parental males and females of both generations at 120 and 360 ppm; similar effects were also seen at higher doses in the preliminary study.

The NOEL and LOEL for reproductive/developmental toxicity that were set by the study authors were appropriate.

D. With the exception of several minor discrepancies between the raw data and the final report, we did not note any major deficiencies that compromised the study.

Item 15--see footnote 1.

16. CBI APPENDIX: Appendix A, Materials and Methods, CBI pp 23-36.

APPENDIX A

Materials and Methods

RIN 5218-93 / TOX Review for Glufosinate Log # 293

Page_____ is not included in this copy.

Pages 297 through 310 are not included.

The material not included contains the following type of information:

____ Identity of product inert ingredients.

____ Identity of product impurities.

____ Description of the product manufacturing process.

____ Description of quality control procedures.

____ Identity of the source of product ingredients.

____ Sales or other commercial/financial information.

____ A draft product label.

____ The product confidential statement of formula.

____ Information about a pending registration action.

__✓__ FIFRA registration data.

____ The document is a duplicate of page(s) _____.

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The information not included is generally considered confidential by product registrants. If you have any questions, please contact the individual who prepared the response to your request.

Reviewed by: Whang Phang, Ph.D. 9/2/88
Secondary Reviewer: Marcia van Gemert, Ph.D. 9/16/88
Robert Zendzian, Ph.D. 9/16/88
Section III, Tox. Branch (TS-769c)

DATA EVALUATION REPORT

006936

STUDY TYPE: Pharmacokinetic study with dermal application

CHEMICAL: HOE 039866; monoammonium [2-amino-4-(hydroxymethyl-phosphinyl)butanoate]; Ignite®

EPA ACCESSION No: 403456-20 CASWELL No.: 580I

EPA ID No: 8340-EO/8340-EI EPA PROJECT No: 8-0146

SPONSOR: Hoechst Celanese Corp.

TESTING LABORATORY: RCC, Umweltchemie AG, P.O. Box CH-4452, Itingen, Switzerland

CITATION: Ellgehausen, H. (1986). Pharmacokinetic study with ^{14}C-Hoe 039866 on various organs/tissues of male rats and excretion pattern of radioactivity after single dermal administration of the test article. RCC, Umweltchemie AG, Switzerland; Report No.: A34502; Project No.: 056597. August 22, 1986. Submitted by Hoechst Celanese Corp., Sommerville, NJ. Aug 27, 1987.

SUMMARY: Groups of male Wistar rats (28/dose) were dermally administered radioactive Hoe 039866 at doses of 0.1, 1.0, and 10 mg/rat on 6 cm^2 of shaved skin. Four rats/dose were exposed for 0.5, 1, 2, 4, 10, 24, & 168 hrs. The test substance was removed from the applicaton site at the end of the exposure period with a soap and water wash except for the 24 and 168 hr groups where the wash was after 10 hrs of exposure. The quantity of radioactivity in feces, urine, and various tissues were measured.

The results indicated that at the low dose 42.5 to 50.8% of the applied radioactivity was absorbed whereas at high dose 26% was absorbed.

After removal and washing of the treated skin a substantial amount of the radioactivity still remained in the skin, and it was gradually absorbed and eliminated.

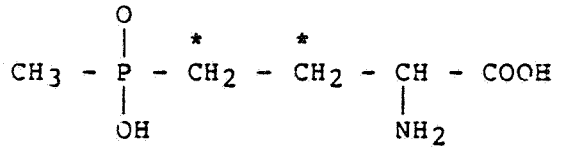
Radioactivity was found in both feces and urine samples, but the majority of Hoe 039866 was eliminated via urine.

In all organs/tissues examined, radioactivity was found to reached a maximum level either at 4 or 10 hrs after exposure. Subsequently, the radioactivity dropped rapidly. The amount of radioactivity found in the brain was very minimal relative to that of kidneys and liver.

This study was well performed and reported. It is classified as Acceptable.

MATERIALS AND METHODS:

Test chemical: ^{14}C-Hoe 039866; radiochemical purity, >98%; specific radioactivity, 43.6 mCi/gm; positions labelled are shown below:



Unlabelled Hoe 039866 (Code: Hoe 039866 OH ZC 95 0001) was used for dilution purposes, and its purity was reported to be 108% (based on chemical analysis at RCC).

The test article was formulated as an aqueous solution using the blank formulation (1.75 gm / 5 ml of water) of Hoe 039866 with code No. Hoe 039866 OH SLOO A401. The stock solutions (5 ml each) for treating the three groups were the following:

Group (mg/rat)	^{14}C-Hoe 039866(mg)	Hoe 039866(mg)	Amount of Radioactivity
2 (0.1)	3.89	-----	0.170 mCi
3 (1.0)	15.42	18.87	0.672 mCi
4 (10.0)	31.11	295.54	1.356 mCi

From these stock solutions, approximately 150 ul were prepared for each rat.

Animals: Male Wistar rats (KFM-WIST outbred, SPF-quality) were obtained from KFM, Kleintierfarm Madoerin AG, CH-4414 Fuellinsforf/Switzerland. These rats weighed 167 - 198 gm.

Experimental Design: Groups of rats (28/dose) were dermally applied (with a brush) radioactive Hoe 039866 at nominal doses of 0.1, 1.0, and 10 mg/rat on 6 cm^2 of shaved skin. The application site was dried and protected with a "non-adsorbing perforated foil which was fixed at its outer sides with a plaster". The treated animals in each dose group were further divided into seven subgroups with 4 rats each. These subgroups were sacrificed at 0.5, 1, 2, 4, 10, 24, and 168 hours (hrs) after treattment. The test article was removed 10 hrs after dosing for rats which were sacrified at 24 and 168 hrs, and for others it was removed at time of sacrifice by washing the treated skin with liquid soap and rinsed with warm water. The amount of radioactivity in blood, feces, urine, liver, brain, kidneys, treated skin, carcass, skin-wash, and cage-wash was determined.

RESULTS:

Absorption: Based on the radioactivity measurements of the samples of blood, liver, kidneys, brain, treated skin, urine, feces, cage-wash, and carcass, the amount of radioactivity absorbed by different dose groups at various time was calculated and presented in Table 1A, 1B, and 1C and in Figure 1.

In 0.1 mg animals, the total amount of ^{14}C-Hoe 039866 absorbed on the average was 44.7% of the applied dose after 10 hrs and reached a plateau at approximately 49% at 24 hrs (Table 1A & Figure 1).

The 1.0 mg rats absorbed ^{14}C-Hoe 039866 in a similar fashion as that seen in 0.1 mg rats up to 10 hrs after dosing; Beyond 10 hrs, the absorptions decreased (Table 1B and Figure 1).

For rats in 10 mg group, the total percent of ^{14}C-Hoe 039866 absorbed was less than those of the two lower dose groups at all examination times (Table 1C & Figure 1). A maximum absorption of 39% of the administered radioactivity was approached at approximately 10 hrs, after which the absorption decreased.

Excretion:

In general radioactivity found in the urine was in greater quantity than that seen in the feces at any sacrifice interval and for any dose groups (Table 1). Based upon the data on animals which were sacrificed at 168 hrs after dosing, the major portion of the radioactivity absorbed was excreted within the first 24 hrs at all dose levels (Table 2). Similar excretion pattern was seen in feces as that in urine of all dosed animals (Table 2).

Radioactivity in tissues:

In general, there appeared to be a dose-related increase in the residual radioactivity in the tissues examined.

Treated skin: After washing of the treated skin, more radioactivity remained in the treated skin than in any other tissue in all dose groups at each sacrifice interval (Table 3). The radioactivity remaining in the treated skin was shown to be gradually absorbed with time (Table 1 & 3). As an example in 10 mg animals, 5.85% of the applied radioactivity was found in the treated skin after 10 hrs; after 24 and 168 hrs the radioactivity decreased to 3.74 and 0.77%, respectively. This finding indicated that the radioactivity remaining in the treated skin was slowly absorbed and eliminated.

Brain: The radioactivity in the brain was found to be rather low in all treated animals at all sacrifice intervals (Table 3A & Figure 2). In some instances the levels were below that of the background (Table 3B).

Blood: The highest radioactivity level in the blood was found to be 0.3, 0.17, and 1.62 ug ^{14}C-Hoe 039866 equivalent/g tissue at 4 hrs after treatment for 0.1, 1.0, and 10 mg dose groups, respectively (Table 3A). After 4 hrs, a rapid decline in radioactivity level in the blood was found in all dose levels (Figure 3).

Liver: The radioactivity levels in the liver reached a maximum after 10 hrs amounting to 0.09, 0.68, and 4.68 ug equivalent/g tissue for 0.1, 1.0, and 10.0 mg groups, respectively (Table 3 & Figure 4). After 168 hrs, radioactivity level in the liver dropped rapidly (0.05, 0.24, & 0.39 ug equivalent/g tissue for 0.1, 1.0, 10.0 mg groups, respectively).

Kidneys: After 4 hrs the radioactivity levels in the kidneys reached a maximum representing 1.13, 5.79, & 35.88 ug equivalent/g tissue for 0.1, 1.0, and 10.0 mg groups (Table 3 & Figure 5). After 10 hrs, the radioactivity level decreased drastically (Figure 5).

Carcass: In the carcass, radioactivity level reached the highest level after 10 hrs in all dose groups (Table 3). As in all other tissues, the level dropped rapidly thereafter.

DISCUSSION:

Dermal application of radioactive Hoe 039866 at amounts of 0.1, 1.0, and 10.0 mg resulted in an absorption on the average of 44.7% of the applied radioactivity at low dose group; 25%, for high dose animals.

Urinary excretion was found to be the major route of elimination, and a significant amount of radioactivity was also excreted in the feces.

Analysis of the radioactivity in different organs indicated that a very small amount of radioactivity was found in the brain relative to other organs. After removal and washing of the treated skin a substantial amount of the radioactivity still remained in the skin, and it was gradually absorbed and eliminated. In the blood after 4 hrs after treatment, the radioactivity reached the highest level; subsequently the concentration declined rapidly. In the kidneys, after 4 hrs the radioactivity reached a maximum level and dropped rather quickly thereafter. In the liver, the radioactivity reached the highest level after 10 hrs and subsequently declined rapidly.

Although radioactivity in the carcass increased initially like in other organs, after 168 hrs it dropped drastically.

This study was well performed and reported. It is classified as Acceptable.

RIN 5218-93 / Tox Review for Glufosinate Log # 293

Page_____ is not included in this copy.

Pages 316 through 323 are not included.

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____ The product confidential statement of formula.

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Reviewed by: Whang Phang, Ph.D. 8/31/88
Secondary Reviewer: Marcia van Gemert, Ph.D. 8/31/88
Section III, Tox. Branch (TS-769c)

006936

DATA EVALUATION REPORT

STUDY TYPE: Metabolism: Single oral Dose (Rat)(30 mg/kg)

CHEMICAL: HOE 039866; monoammonium [2-amino-4-(hydroxymethyl-phosphinyl)butanoate]; Ignite®

EPA ACCESSION No: 403456-40 CASWELL No.: 580I

EPA ID No: 8340-EO/8340-EI EPA PROJECT No: 8-0146

SPONSOR: Hoechst Celanese Corp.

TESTING LABORATORY: Hoechst AG, 6230 Frankfurt AM Main 80
Federal Republic of Germany

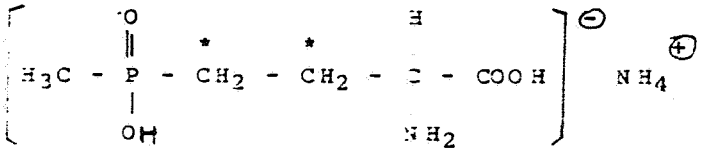
CITATION: Kellner, H, -M. and Eckert, H. G. (1985). Hoe 039866-^{14}C: Studies of kinetics and residue determinations in rats following oral administration of 30 mg/kg body weight. Hoechst AG, Germany; Report No.: A33239; Project No.: 01-L42-0467-85. Nov 13, 1985. Submitted by Hoechst Celanese Corp., Sommerville, NJ. Aug 27, 1987.

SUMMARY: Oral administration of a nominal dose (30 mg/kg) of ^{14}C-Hoe 039866 to groups of Wistar rats (5/sex) resulted in rapid elimination during the first 24 hrs for both males and females. The major route of excretion was via feces (88% and 84% of the administered radioactivity for males and females, respectively). Within 7 days of post dosing, greater than 94% of the dose was eliminated. Kinetics analysis indicated that the process of excretion was a two-phrase process. The tissue radioactivity level for kidneys, liver, and gonads was just above the background level.

Although this study did not identify any metabolite, the report presented valid kinietics data. It is classified as Minimum.

MATERIAL AND METHODS:

Test article: ^{14}C-Hoe 039866 with batch No.: 11076 II was diluted with the unlabelled Hoe 039866 (Code No.: Hoe 039866 OH ZB 99 002) in a ratio of 1 : 10 until a radioactivity of approximately 2.0 mCi/g was achieved. The test article was dissolved in distilled water. The radiopurity of the test material was 98%.



Test animals: Wistar rats were obtained from Hoechst AG breeding colony WISKf (SPF 71). These rats were approximately 12 weeks (wks) old, and weighed 160 gm for all females and 4/5 males (Table 1, taken from page 15 of the report). It is highly unusual to to have two groups of animals with such homogenous body weights.

Experimental procedures:

Groups of animals (5/sex) were administered (by gavage) the test article at a nominal dose of 30 mg/kg. The treated animals and two controls (1/sex) were individually kept in "the metabolic cages with a device for separating the urine from the faeces". Samples of urine and feces were collected, and 7 day after dosing the animals were sacrificed. Liver, kidneys, spleen, gonads, heart, lung, skeletal muscle, subcutaneous fat, renal fat, brain, bone, and blood, were collected for analysis of radioactive residue. The methods for processing the tissue samples and chemical analyses are excerpted from the report and presented in the Appendix.

RESULTS:

Excretion: Majority of the administered radioactivity in both males and females was excreted during the first 24 hours (hrs) post dosing (Tables 2 and 3, taken from pages 16 & 17 of the report, respectively). The major route of excretion was in the feces for males and females and amounted to 88% and 84% of the administered radioactivity, respectively (Table 4, taken from page 18 of the report). A small percentage of the radioactivity was excreted via urine by both male and female rats (Table 4). Greater than 94% of the administered radioactivity was excreted within 7 days of post dosing.

Figures 1 and 2 showed that excretion of Hoe 039866 was a 2-phase process in feces or urine (Figures 1 & 2 are taken from pages 24 & 25 of the report). The half lives of excretion were summarized in Table 5 (taken from page 19 of the report). For urinary excretion, the biological half-live ($t_{1/2}$) for phase I was 7.3 and 6.3 hrs for males and females, respectively. For fecal excretion, it was 4.6 and 6.0 for males and females, respectively (Table 5). The $t_{1/2}$ for phase II was substantially greater.

Residues in tissues: The amount of radioactivity remaining in various tissues and organs was less than 0.1% of the administered dose (Table 4, taken from page 18 of the report). The individual animal data indicated that among the organs examined liver, kidney, and gonad contained levels of radioactivity barely above the detection limit.

Total recovery of the administered radioactivity was 95% for males and 96% for females.

DISCUSSION:

Oral administration of a nominal dose (30 mg/kg) of ^{14}C-Hoe 039866 to groups of Wistar rats (5/sex) resulted in rapid elimination during the first 24 hrs for both males and females. The major route of excretion was via feces (88% and 84% of the administered radioactivity for males and females, respectively). Within 7 days of post dosing, greater than 94% of the dose was eliminated. Kinetics analysis indicated that the process of excretion was a two-phase process. The tissue radioactivity level for kidneys, liver, and gonads was just above the background level.

The study provides useful information, and the data of this study were consistent with those of another study which used lower dose (2 mg/kg)(Hoechst Report No.: A33895). The data of both studies indicated that majority of the administered Hoe 039866 was eliminated within the first 24 hrs, and the major route of excretion was via feces. Very little radioactivity was found in the tissue.

The current study did not identify any metabolites, but the kinetics analysis was valid. The study is classified as Minimum.

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Pages 327 through 334 are not included.

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Reviewed by: Whang Phang, Ph.D. 8/31/88
Secondary Reviewer: Marcia van Gemert, Ph.D. 8/31/88
Section III, Tox. Branch (TS-769c)

006936

DATA EVALUATION REPORT

STUDY TYPE: Metabolism: Repeated Oral Administration (2 mg/kg) (Rats)

CHEMICAL: HOE 039866; monoammonium [2-amino-4-(hydroxymethyl-phosphinyl)butanoate]; Ignite®

EPA ACCESSION No: 403456-42 CASWELL No.: 580I

EPA ID No: 8340-EO/8340-EI EPA PROJECT No: 8-0146

SPONSOR: Hoechst Celanese Corp.

TESTING LABORATORY: Hoechst AG, 6230 Frankfurt AM Main 80 Federal Republic of Germany

CITATION: Kellner, H, -M. and Eckert, H. G. (1985). Hoe 039866-^{14}C: Studies of kinetics and residue determinations in rats after repeated oral doses of 2 mg/kg body weight on 15 consecutive days. Hoechst AG, Germany; Report No.: A33975; Project No.: 01-L42-0465-85. Nov 8, 1985. Submitted by Hoechst Celanese Corp., Sommerville, NJ. Aug 27, 1987.

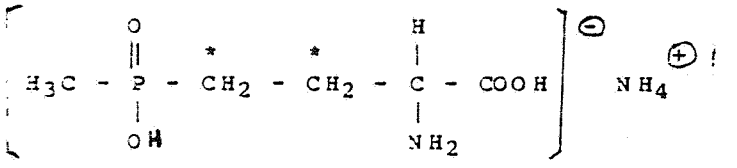
SUMMARY:

Groups of Wistar rats (6/sex) were orally administered (gavage) unlabelled Hoe 039866 for 14 days and ^{14}C-Hoe 039866 at the 15th day at a nominal dose of 2 mg/kg. Majority of the radioactivity was excreted within 24 hrs after the last dose. The major route of elimination was via feces. There was also a two-phase elimination process. More radioactivity was found in the tissues of animals dosed repeatedly than that of animals receiving a single dose.

Although the study did not present any findings on metabolites, it reported valid and useful information on the kinetics of the test agent in rats. This study is classified as Minimum.

MATERIAL AND METHODS:

Test article: ^{14}C-Hoe 039866 with batch No.: 11076 II e and radiochemical purity of 97% was used. Specific radioactivity was 21.87 mCi/g. Positions of radiolabel are shown below:



Unlabelled and labelled Hoe 039866 were individually dissolved in water.

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Test animals: Wistar rats were obtained from breeding colony WISKf (SPF 71) of Hoechst AG. These rats were 10 - 12 weeks old and weighed 240 - 265 g (males) and 210 - 235 g (females).

Experimental procedures: Groups of Wistar rats (6/sex) were orally administered (by gavage) unlabelled Hoe 039866 for 14 days at a nominal dose of 2 mg/kg, and at 15th day 2 mg/kg of ^{14}C-Hoe 039866 was given. One rat/sex was set aside as the control. After the last treatment, the urine and feces samples were collected for 7 days. At 7th day post treatment, the animals were sacrificed. The residual radioactivity in spleen, kidneys, gonads, liver, heart, lungs, skeletal muscle, subcutaneous fat, retroperitoneal fat, brain, bone, carcass, blood, and plasma was determined. The analytical procedures for radioactivity are excerpted from pages 11 and 12 of the report and presented in the Appendix.

RESULT:

Excretion: After 15 days of treatment with 2 mg/kg Hoe 039866, an average of 91% and 89% of the administered radioactivity was excreted in the feces of male and female rats, respectively (Tables 1 & 2, taken from pages 16 & 17 of the report). Majority of the radioactivity was excreted during the first 24 hours (hrs) post dosing (78.5% and 81.5% of the administered dose in males and females, respectively). An average of 9.0% and 8.3% of the administered radioactivity was excreted in the urine of males and females, respectively. Based upon these findings the major route of excretion was via feces.

The kinetics analysis indicated that the elimination of Hoe 039866 was a two-phase process (Figures 1 & 2, taken from pages 24 & 25 of the report) in both urine and feces.

Radioactivity in tissues and organs:

In treated male rats, detectable amounts of radioactivity were found in kidneys, testes, liver, and carcass. Although radioactivity was found in similar organs in females, the amount was substantially less than that seen in males (average % of the administered radioactivity: males, 1.34%; females, 0.14%) (Table 3, taken from page 18 of the report).

DISCUSSION:

With repeated oral dosing (2 mg/kg) for 15 days, majority of the radioactivity was excreted in the feces by both males and

females (males, 91%; females, 89%). Major route of excretion was via feces, and there was no sex difference in excretion. Relative to single dose animals, more radioactivity was found in the organs and tissues of animals which received repeated doses of the test article.

Although the study did not identify the metabolites in urine, feces, or organs, the report presented valid kinetics data. It is classified as Minimum.

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Reviewed by: Whang Phang, Ph.D. 8/2/88
Secondary Reviewer: Marcia van Gemert, Ph.D. 8/4/88
Section III, Tox. Branch (TS-769c)

DATA EVALUATION REPORT

006936

STUDY TYPE: Metabolism (Dogs)

CHEMICAL: HOE 039866; monoammonium [2-amino-4-(hydroxymethyl-phosphinyl)butanoate]; Ignite®

EPA ACCESSION No: 403456-21 CASWELL No.: 580I

EPA ID No: 8340-EO/8340-EI EPA PROJECT No: 8-0146

SPONSOR: Hoechst Celanese Corp.

TESTING LABORATORY: RCC, Umweltchemie AG, Switzerland

CITATION: Ellgehausen, H. (1986). Pharmacokinetic study with ^{14}C-Hoe 039866 on various organs of male and female Beagle dogs and excretion pattern of radioactivity after single oral administration of the test article. RCC, Umweltchemie AG, Switzerland; Report No.: A34282; Project No.: 050185; April 28, 1986. Submitted by Hoechst Celanese Corp., Sommerville, NJ. Aug 27, 1987.

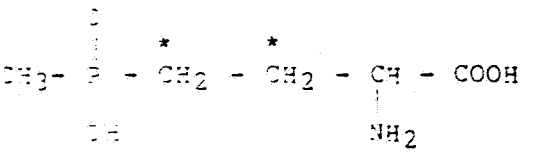
SUMMARY: Two Beagle dogs per sex were orally administered (capsule) ^{14}C-Hoe 039866 as a single dose (8 mg/kg). One dog/sex was sacrificed at 6 hours after administration of the test agent, and the others were sacrificed at 24 hours. The results indicated that a small quantity of Hoe 039866 was absorbed, and the major route of excretion was via feces. The test agent was found in the blood, plasma, heart, liver, kidneys, and in various areas of the brain. Much of the excreted compounds consisted of the unmetabolized parent chemical, Hoe 039866. Only a single metabolite, Hoe 061517 was found in the urine.

This study has many deficiencies and is considered as Supplementary.

METHODS AND MATERIALS:

1). Test compound: Unlabelled Hoe 039866, technical 95.3% purity; Code: Hoe 039866 OH ZC95 0001

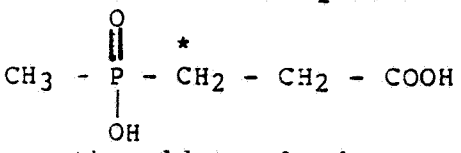
Radiolabelled Hoe 039866 was as follows:
Radiochem. purity: 98%
Positions labelled:



Specific radioactivity: 54.08 mCi/g

Reference compounds: Analytical grade unlabelled Hoe 039866
Code: Hoe 039866 OH ZB 98 0001

Analytical grade of unlabelled and labelled Hoe 061517 (metabolite) with specific radioactivity of 24.8 mCi/g
Code: Hoe 061517 OQ ZC 99 0001



2). Animals: Four to 6 month old Beagle dogs weighing 4.8-7.1 kg; these animals were obtained from KFM, Kleintierfarm, Madoerin AG, Switzerland.

3). Experimental Methods: The details of the experimental procedures were excerpted from the submitted report and presented in the Appendix.

Briefly, 2 dogs/sex were orally administered (by capsule) a single dose (8 mg/kg) of ^{14}C-Hoe 039866. At 6 or 24 hours (hrs) after dosing 1 dog/sex was sacrificed, and blood and tissue samples were collected. Samples of urine and feces were collected from 0-6 hrs and 6-24 hrs after dosing. Cage washes and samples of blood, tissues, urine, and feces were measured for radioactivity. In addition, samples of feces, urine, and tissues were analyzed for any metabolite present.

RESULTS:

It should be noted that the results in the submitted report were in the form of individual animal data.

Excretion: In urinary samples of the treated dogs, there was a small quantity of radioactivity found in 6 and 24 hrs after administration of test article (ranging from 2.79 to 6.93% of the administered radioactivity)(Table 1).

In feces, there was a substantial variation in amount of radio activity found in the samples of 6 or 24 hrs (Table 1).

In general, the amount of radioactivity excreted in 24 hrs by both male and female dogs was approximately 94% of the administered dose (Table 1).

Radioactivity in tissues:

Blood (Table 2):

In the two treated male dogs, maximum level of radioactivity was reached at approximately 2 hrs after dosing. In females, the maximum level was found to be at 4 hrs after treatment.

Plasma (Table 2):

The radioactivity level in plasma of both treated males and females followed similar pattern as those in the blood, except there was more radioactivity detected in plasma at most examination times.

Based upon the radioactivity in the blood of a male and female dog, the author of the report calculated the half-life of the test article in the blood to be 9.2 hrs in male dog and 1.7 hrs in female dog for the first 4 hrs after compound administration. It should be emphasized that the calculation of half-life was conducted using the data from one animal only.

Brain (Table 3a & 3b):

In treated male and female dogs, the radioactivity levels in cortex, cerebellum, midbrain, brain stem, and spinal cord were measured and found to be substantially greater than that seen in the background radioactivity levels (Table 3b).

Heart, Liver, and Kidneys (Table 3a & 3b)

Relative to the background radioactivity levels, the amount of radioactivity in the heart, liver, and kidney of all treated dogs was markedly increased, especially that in the liver and kidneys (Table 3a).

METABOLITES (Tables 4, 5, 6, & 7)

In urine, unmetabolized Hoe 039866 accounted for the majority of the excreted radioactivity (Table 4). A metabolite, Hoe 061517, was found in a small quantity. The structure of Hoe 061517 is presented in the Methods and Material section of this DER.

In feces of all treated dogs, radioactivity was found in an extractable and an unextractable fraction (Table 5). In the extractable fraction unmetabolized Hoe 039866 was found to be the main constituent, and Hoe 061517 was not detected. Similar patterns were found in liver, kidneys, and blood (Tables 6 & 7).

DISCUSSION:

Based upon the data presented, a small quantity of Hoe 039866 was absorbed with oral administration, and the major route of excretion was via feces. The test agent was found in the blood, plasma, heart, liver, kidneys, and in various areas of the brain. Much of the excreted compounds consisted of the unmetabolized parent chemical, Hoe 039866. Only a single metabolite, Hoe 061517 was found in the urine.

Although the study provides useful information, the report has many deficiencies which include:

1). The half life of the test compound was calculated from the data of a single animal instead of from the means of several animals.
2). The amount of radioactivity in fat should also be examined.
3). The quantity of radioactivity in various tissues should be reported in terms of administered dose.

The study does not meet the requirements of the Guidelines and is considered Supplementary.

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Reviewed by: Whang Phang, Ph.D. 8/31/88
Secondary Reviewer: Marcia van Gemert, Ph.D. M. van Gemert 8/31/88
Section III, Tox. Branch (TS-769c)

006936

DATA EVALUATION REPORT

STUDY TYPE: Metabolism (Dogs)

CHEMICAL: HOE 039866; monoammonium [2-amino-4-(hydroxymethyl-phosphinyl)butanoate]; Ignite®

EPA ACCESSION No: 403456-44 CASWELL No.: 580I

EPA ID No: 8340-EO/8340-EI EPA PROJECT No: 8-0146

SPONSOR: Hoechst Celanese Corp.

TESTING LABORATORY: Hoechst AG, 6230 Frankfurt AM Main 80
Federal Republic of Germany

CITATION: Ellgehausen, H. (1986). Absorption, excretion, and metabolism of Hoe 039866 by Beagle dogs after repeated administration of unlabelled test article followed by repeated administration of ^{14}C-labelled test article. RCC, Umweltchemie AG, Switzerland; Report No.: A33914; Project No.: 048734. March 20, 1986. Submitted by Hoechst Celanese Corp., Sommerville, NJ. Aug 27, 1987.

SUMMARY: Groups of Beagle dogs were orally administered (by capsule) unlabelled Hoe 039866 for the first 18 days and subsequently ^{14}C-Hoe 039866 for 10 days. The animals were sacrificed at various hours after the last dose. The results indicated that a small proportion of the administered dose was absorbed. At a dose of 8 mg/kg, radioactive Hoe 039866 was found to enter the brain and still could be detected 4 days after the last administration. Radioactivity was also found in kidneys and livers. The major route of excretion appeared to be via feces, and the main excreted compound was the parent compound in either feces or urine. In urine a metabolite, Hoe 061517 was found.

The study has many deficiencies and is classified as Supplementary.

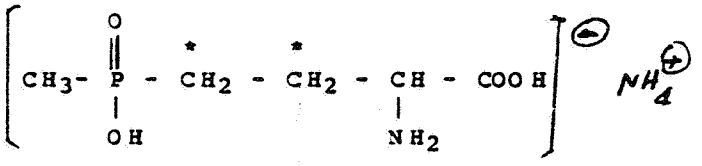
METHODS AND MATERIALS:

1). Test compound: Unlabelled Hoe 039866, technical 95.3% purity; Code: Hoe 039866 OH ZC95 0001

Radiolabelled Hoe 039866 was as follows:
Radiochem. purity: 98%

368

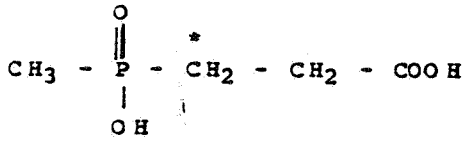
Position of labelled:



Specific radioactivity: 54.08 mCi/g

Reference compounds: Analytical grade unlabelled Hoe 039866
Code: Hoe 039866 OH ZB 98 0001

Analytical grade of unlabelled and labelled Hoe 061517 (metabolite)
Code: Hoe 061517 OQ ZC 99 0001



2). Animal: Four to 7 month old Beagle dogs weighing 3.7 to 7.3 kg were obtained from KMF, Kleintierfarm Madoerin AG, Switzerland.

3). Experimental procedures: The details of the experimental protocol are excerpted from the submitted report and presented in the Appendix.

Briefly, groups of dogs (6/sex/dose) were orally administered (by capsule) unlabelled Hoe 039866 for the first 18 days. From days 19-28, the test animals were treated with ^{14}C-Hoe 039866. The doses of the test agent were 1 and 8 mg/kg, and a group of control animals was also included. Samples of blood, urine, and feces were collected for analyses of the amount of radioactivity and metabolite in these samples. One animal/sex/dose was sacrificed at various times after the last treatment. Amount of radioactivity in the heart, liver, kidney, and in different parts of the brain was analyzed.

RESULTS:

It should be noted that the data presented in this report were not in the form of the means or averages; they were mainly as individual animal data.

Absorption: The absorption aspect of this study was not adequately conducted. However, based upon the measurements of radioactivity in blood and plasma at various intervals after dosing and the data on excretion and metabolism, the test article did not appear to be absorbed readily (Tables 1 & 2).

Excretion: Much of the administered radioactivity was excreted in feces (average 81%) of all treated dogs (Table 1). Approximately 14 to 17 % of radioactivity was found in the urine of both males and females of the 2 dose groups. Ninty-six hours after the last treatment, the total recovery of the radioactivity was greater than 96% of the applied dose in all treated dogs.

The author of the report selected the data on the radioactivity in plasma and blood at different times of a male dog (No. 13) and calculated the half life of the test agent (Table 2). For a male dog the half-life was calculated to be 46.2 hrs in blood and 16.1 hrs in plasma.

Distribution: A thorough analysis of tissue distribution of the test agent was not carried out. The individual animal data indicated that brain stem, cortex, cerebellum, mid brain, and spinal cord of high dose dogs which were sacrificed 96 hours after the last treatment still contained radioactivity (Table 3, a representative set of individual animal data). However, the means or averages of the radioactivity in different organs were not calculated. Kidneys and liver of the treated dogs also contained radioactivity (Table 3).

Metabolites: Based upon the report, the chemical analyses of metabolite in the samples of tissues, urine, and feces from 1 dog/sex/dose were carried out (Table 4). The limited data indicated that unmetabolized Hoe 039866 accounted for 78 to 100% of of the urinary radioactivity or 2 to 3% of the administered dose. A metabolite Hoe 061517 accounted for 11 to 24% of the radioactivity in the urine and 0.3 to 0.6% of the administered dose.

In feces collected 0-96 hours after the last treatment, radioactivity was found in "extractable" and "unextractable" fractions. The radioactivity in the "extractable" fraction was 72 to 86% of the "recovered" radioactivity, and that in the "unextractable" fraction was 13 to 18%. In the "extractable" fraction Hoe 039866 accounted for 81 to 87% of the "recovered" radioactivity, and a metabolite Hoe 061517 was either not analyzed or not detected (Table 5). In feces Hoe 039866 was calculated to be less than 4% of the administered radioactivity.

Both Hoe 039866 and Hoe 061517 were found in liver and kidneys of high dose male and female dogs (Table 6). In kidneys of males and females, Hoe 061517 was found to be in larger amount than that of the parent compound. However, in the liver, both the parent compound and the metabolite appeared to be presented in similar quantity in some dogs and varied greatly in others. For proper evaluation of the results, the author should have presented the quantity of the metabolite and parent compound in different tissues as percentage of the administered dose.

DISCUSSION AND CONCLUSION:

Based upon the data presented in this report, a small proportion of the administered dose was absorbed. At a dose of 8 mg/kg, radioactive Hoe 039866 was found to enter the brain and still could be detected 4 days after the last administration. Radioactivity was present in kidneys and livers. The major route of excretion appeared to be via feces, and the major excreted compound was the parent compound in either feces or urine. In urine a metabolite, Hoe 061517 was found.

Although the study provides useful information, the report has many deficiencies which include:

1). No means or averages of any parameters measured was presented. Essentially all the data presented were on individual animals.

2). The half-life of the test compound should be calculated from the data of several animals instead of a single animal.

3). The amount of radioactivity in fat should also be examined.

4). The percentage of the administered radioactivity in the kidneys and liver should also be calculated.

The study does not meet the requirements of the Guidelines and is considered Supplementary.

RIN 5218-93 / Tox Review for Glufosinate Log # 293

Page_____ is not included in this copy.

Pages 372 through 388 are not included.

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____ Identity of product inert ingredients.

____ Identity of product impurities.

____ Description of the product manufacturing process.

____ Description of quality control procedures.

____ Identity of the source of product ingredients.

____ Sales or other commercial/financial information.

____ A draft product label.

____ The product confidential statement of formula.

____ Information about a pending registration action.

__✓__ FIFRA registration data.

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Reviewed by: Whang Phang, Ph.D. 9/14/88
Secondary Reviewer: Marcia van Gemert, Ph.D.
Section III, Tox. Branch (TS-769c) 9/15/88

006936

DATA EVALUATION REPORT

006936

STUDY TYPE: Metabolism: Repeated Dosing (10 & 100 mg/kg)(Rats)

CHEMICAL: HOE 039866; monoammonium [2-amino-4-(hydroxymethyl-phosphinyl)butanoate]; Ignite®

EPA ACCESSION No: 403456-43 CASWELL No.: 580I

EPA ID No: 8340-EO/8340-EI EPA PROJECT No: 8-0146

SPONSOR: Hoechst Celanese Corp.

TESTING LABORATORY: Hoechst AG, 6230 Frankfurt AM Main 80
Federal Republic of Germany

CITATION: Schwalbe-Fehl, M., Steinau, M., Scheinkonig, U., Kellner, H. -M., and Eckert, H. G. (1985). Hoe 039866-^{14}C: Metabolism and residue determinations in female rats after repeated oral administration of 10 and 100 mg/kg body weight/day, respectively. Hoechst AG, Germany. Hoechst Report No.: A33268; Project No.: CM007/85. May 13, 1986. Submitted by Hoechst Celanese Corp., Sommerville, NJ. Aug 27, 1987.

SUMMARY:

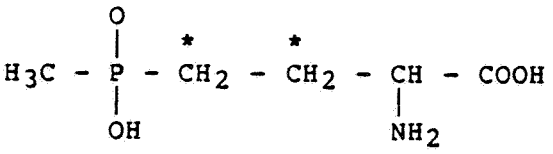
When groups of female Wistar rats were repeatedly treated (by gavage) with Hoe 039866 at doses of 10 or 100 mg/kg, the major route of elimination was via feces. Unchanged test compound accounted for the majority of the radioactivity in urine, feces, kidneys, liver, spleen, and brain. Metabolite, Hoe 061517 was found in urine, feces, and organs. Another metabolite Hoe 086486 was detected in some urine and feces samples of 100 mg/kg animals. Repeated oral dosing with the test agent did not influence the pattern of excretion and residue accumulation in different organs.

This study provides useful information concerning metabolism and excretion of the test agent. However, the report presents only summary data which could not be verified. In addition, the study design calls for the pooling of all the urine or feces samples in a group of animals. This type of experimental design is not acceptable. Therefore, this study is classified as Supplementary.

MATERIALS AND METHODS

Test chemical: Unlabelled Hoe 039866 (Code No.: Hoe 039866 OH ZB98 0001); purity, 99.6%

^{14}C-Hoe 039866: The radiolabelled chemical was kept on stock as a free acid (^{14}C-Hoe 035956). The structure of the ^{14}C-free acid is shown below:

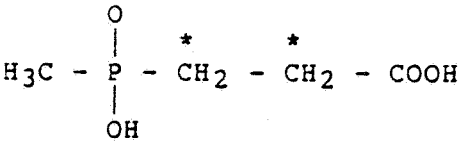


^{14}C - Hoe 035956

The free acid was readily converted to ^{14}C-Hoe 039866 by dissolving it in water and adding an equimolar amount of aqueous ammonia.

The test article was prepared by mixing one part of ^{14}C-Hoe 039866 with 10 parts of unlabelled Hoe 039866. The mixture yielded specific activities of 8784 dpm/ug and 8478 dpm/ug for 10 mg/kg and 100 mg/kg dose levels, respectively.

Reference Compound: ^{14}C-Hoe 061517 was a known metabolite of Hoe 039866 in animals, plants, and soil. The structure is presented below:



3-methylpnosphinico-propionic acid

Test Animals: Thirty SPF-Wistar rats were obtained from Hoechst WISKf (SPF 71) breeding colony. These rats were approximately 10-12 weeks old and weighed 170-220 gm.

Treatment of test animals: Animals were orally given the test article at the 1st, 8th, 9th, and 10th administrations, and all other administrations were with non-labelled Hoe 039866. Using this scheme of administration, 15 females received 10 mg/kg, and another 15 females were treated with 100 mg/kg. The animals in each dose group were further divided into four subgroups according to the following times of sacrifice:

	Group A	Group B	Group C	Group D†
No. of rats per group	2	5	5	3
Sacrifice hrs. after last treatment	24 (1)*	24 (10)*	48 (10)*	24 (10)*

* The number in the parenthesis indicates the last treatment prior to sacrifice.
† This group of animals was designated for the determination of the amount of ^{14}C-Hoe 039866 in blood.

The details for sampling excreta are excerpted from the report and presented in Appendix 1. Briefly, samples of urine were collected from each subgroup at various time intervals, and the sample were then pooled. Similar sampling procedures were used for feces.

Blood samples were collected at 0, 1, 2, 4, 6, 8, and 24 hrs after treatment with ^{14}C-Hoe 039866.

At sacrifice, liver, kidneys, spleen, and brain were removed. Each organ from a group was pooled and analyzed for the amounts radioactivity and metabolites.

The methods of measuring the radioactivity and the determinations of the metabolites are excerpted from the report and presented in the Appendix 2.

RESULTS

Excretion: Majority of the radioactivity was excreted in the feces during the first 24 hrs after treatment (Table 1, taken from page 29 of the report). The data also indicated that repeated dosing did not influence the excretion pattern. Urinary excretion was a minor route of elimination (Table 1). There seemed to be a large difference in the amount of radioactivity in the 0-24 hr samples between Group A and Group B even though all the samples were taken after the 1st radiolabelled dose. An explanation should be offered for this difference. The report must also present individual sample determinations for verification and proper analysis of the data.

Radioactivity in organs: Less than 0.1% of the administered radioactivity was found in liver, kidneys, spleen, and brain of either 10 or 100 mg/kg rats (Table 2, taken from page 31 of the report).

Metabolites: In urine, unchanged ^{14}C-Hoe 039866 accounted greater than 70% of the radioactivity in 10 or 100 mg/kg rats (Table 3, taken from page 32 of the report). The metabolite, Hoe 061517, was seen in some urine samples of 10 mg/kg rats, and the amount ranged from 2 to 21% of the radioactivity. In contrast, Hoe 061517 was present in all samples of 100 mg/kg rats; it accounted for greater than 20% of the radioactivity. Another metabolite, Hoe 086486 was found only in a few samples.

In feces, unchanged Hoe 039866 accounted for greater than 92% of the radioactivity in the fecal samples of 100 mg/kg animals (Table 4, taken from page 33 of the report). The metabolite, Hoe 061517 amounted to a range of 3-5% of the radioactivity. Hoe 086486 was seen only in a few samples. No data on the metabolite in feces of 10 mg/kg rats were reported.

Unchanged Hoe 039866 was present in brain, spleen, liver, and kidneys, and it accounted for 68 to 80% of the radioactivity in these organs (Table 5, taken from page 34 of the report). Hoe 061517 was also present, and it only amounted to 20 to 32% of the radioactivity in these organs.

The total recoveries expressed as percentage of the administered radioactivity were 77, 94, and 106% for Groups A, B, and C of 10 mg/kg rats, respectively; for 100 mg/kg rats, they were 98, 106, and 108% for Groups A, B, and C, respectively (Table 6, taken from page 30 of the report).

DISCUSSION:

When groups of female Wistar rats were repeatedly treated with Hoe 039866 at doses of 10 or 100 mg/kg, the major route of elimination was via feces. Unchanged test compound accounted for the majority of the radioactivity in urine, feces, kidneys, liver, spleen, and brain. Metabolite, Hoe 061517 was found in urine, feces, and organs. Another metabolite Hoe 086486 was detected in some urine and feces samples of 100 mg/kg animals. Repeated oral dosing with the test agent did not influence the pattern of excretion and residue accumulation in different organs.

This study provides useful information concerning metabolism and excretion of the test agent. However, the report presents only summary data which could not be verified. In addition, the study design calls for the pooling of all the urine, feces, or organ samples in a group of animals. This type of experimental design is not acceptable. Therefore, this study is classified as Supplementary.

RIN 5218-93 / Tox Review for Glufosinate Log #293

Page_____ is not included in this copy.

Pages 393 through 402 are not included.

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____ Identity of product inert ingredients.

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Reviewed by: Whang Phang, Ph.D. 8/29/88
Secondary Reviewer: Marcia van Gemert, Ph.D. 8/31/88
Section III, Tox. Branch (TS-769c)

DATA EVALUATION REPORT

006936

STUDY TYPE: Metabolism: Single oral Dose (Rat)(800 mg/kg)

CHEMICAL: HOE 039866; monoammonium [2-amino-4-(hydroxymethyl-phosphinyl)butanoate]; Ignite®

006936

EPA ACCESSION No: 403456-37 CASWELL No.: 580I

EPA ID No: 8340-EO/8340-EI EPA PROJECT No: 8-0146

SPONSOR: Hoechst Celanese Corp.

TESTING LABORATORY: Hoechst AG, 6230 Frankfurt AM Main 80
Federal Republic of Germany

CITATION: Schwalbe-Fehl, M., Steinau, M., Kellner, H, -M. and Eckert, H. G. (1985). Hoe 039866-^{14}C: Metabolism and residue determinations in rats after single oral administration of 800 mg/kg body weight. Hoechst AG, Germany. Hoechst Report No.: A32953; Project No.: CM008/85. Dec 17, 1985. Submitted by Hoechst Celanese Corp., Sommerville, NJ. Aug 27, 1987.

SUMMARY: Groups of Wistar rats (3/sex) were administered (by gavage) ^{14}C-Hoe 039866 at a dose of 800 mg/kg. Three treated rats were sacrificed at 6 and 24 hrs after treatment. Samples of the urine, feces, liver, kidneys, spleen, and brain were pooled according to sex and time of sacrifice. The results indicated that in urine, unmetabolized Hoe 039866 and two other metabolites, Hoe 061517 and Hoe 086486 were found. In feces, only unmetabolized Hoe 039866 was found.

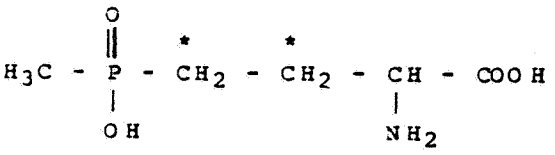
The report presents useful information concerning the metabolites of the test article; however, the experimental procedures applied the pooling of the samples of all animals in each sex, and the results as presented in the report are summary data which can not be verified by individual animal data or determinations. In addition, the number of test animals/sex (3/sex) is insufficient in this study. Therefore, this study is classified as Supplementary.

MATERIALS AND METHODS:

Test Article:

1). Unlabelled Hoe 039866: Code No. Hoe 039866 OH ZB 99 0002; purity, 99.5%.
2). Labelled substance: Code No. Hoe 035956 OT ZE98 0005
Radiochemical purity: 98%
specific radioactivity: 54.08 mCi/g

The labelled test substance was stored as a free acid the structure of which is presented below:

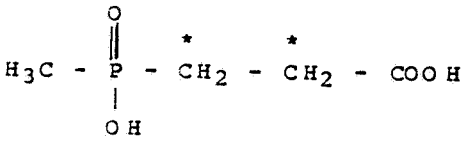


^{14}C - Hoe 035956

The free acid was readily converted to ^{14}C-Hoe 039866 by dissolving it in water and adding an equimolar amounts of aqueous ammonia.

The test article was prepared by mixing one part of ^{14}C-Hoe 039866 with 30 parts of unlabelled Hoe 039866. The mixture yielded a specific activity of 1.58 mCi/g, and it was suspended in 2% (w/w) potato starch. The concentration of the Hoe 039866 in the application mixture was 133.5 mg/g suspension.

Reference Compound: ^{14}C-Hoe 061517 was a known metabolite of Hoe 039866 in animals, plants, and soil. The structure is presented below:



3-methylphosphinico-propionic acid

Test Animals: Nine male and 9 female SPF-Wistar rats were obtained from Hoechst WISKf (SPF 71) breeding colony. These rats were approximately 10-12 weeks old and weighed 160-195 gm.

The test animals were divided into three groups and sacrificed at different times after dosing as shown below:

Group No.	No. of Animals	Sacrifice Time Hours (hrs) After Dosing
1	3/sex	6
2	3/sex	24
3*	3/sex	24

* This group was for the determining the radioactivity in the blood.

Experimental Procedures: Groups of Wistar rats (3/sex) were orally administered the test article at a nominal dose of 800 mg/kg (by gastric tube). The treated animals were individually caged. Samples of urine and feces were collected at 0-6 hrs for group 1 animals and at 0-24 hrs for group 2 animals. From group 3 animals, blood samples were collected at 0.25, 0.5, 1, 2, 4, 6, 7.5, and 24 hrs after dosing. Animals of groups 1 and 2 were sacrificed at 6 hrs and 24 hrs after dosing, respectively. Liver, kidneys, spleen, and brain were removed and pooled according to sex and time of sacrifice. In addition, the samples of the urine and feces were also pooled according to sex and time of sacrifce. The amount of radioactivity and metabolites in all the pooled samples of urine, feces, and organs were measured by scintillation counting and by gas chromatograph/mass spectroscopy, respectively. The details of chemical analyses are excerpted from the report and presented in Appendix 1.

RESULTS:

Radioactivity in urine: In group 1 animals, the amounts of radioactivity found in the urine were 0.5% and 1.2% of the administered dose in males and females, respectively. In group 2 animals, the amounts were 3.9% for males and 3.6% for females.

Metabolites in urine: The report states that there were three radioactive fractions in the urine sample with high performance liquid chromatography (HPLC) analyses. However, this statement is not consistent with a representative HPLC-chromatogram presented in page 29 of the report; a copy of this page is presented in Appendix 2. In this chromatogram there were four peaks identified.

In the urine, there were two identified metabolites, Hoe 061517 and 3-(methylphosphinico)-3-oxo-propionic acid or Hoe 086486, and the unmetabolized Hoe 039866 (Table 1, taken from page 25 of the report).

Feces: Most of the radioactivity in the fecal samples was extracted with hot water. The amount of radioactivity in the feces of group 1 animals were less than 0.001% of the administered dose (Table 2, taken from page 26 of the report), and that of group 2 animals were 21.4% (males) and 23.9% (females). With HPLC analysis, unmetabolized Hoe 039866 was found to account for practically all the radioactivity in the feces.

Organs: The amount of radioactivity found in liver, kidneys, spleen, and brain was less than 0.1% of the administered radioactivity (Table 3, taken from page 27 of the report). Attempts were made to identify the metabolites in these organs; detectable

amounts of unchanged Hoe 039866 were found in all organs examined. In all organs unmetabolized Hoe 039866 represents a major portion of the radioactivity found (Table 4, taken from page 28 of the report). In liver and kidneys, both Hoe 061517 and Hoe 086486 were identified.

Blood: The data on the amount of radioactivity in the blood were not presented in the report; however, the report stated that ^{14}C-Hoe 039866 was absorbed rapidly after oral administration. In males maximum blood concentration was achieved 1 hr after dosing; in females, 0.5 hrs. The half-lives were 5 and 4 hrs for males and females, respectively.

In general, all the data obtained from this study indicated that in rats no sex difference existed in the metabolism of Hoe 039866.

DISCUSSION:

Groups of Wistar rats were administered (by gavage) the test article at a dose of 800 mg/kg. Three treated rats/sex were sacrificed at 6 and 24 hrs after treatment. The times for sacrifice were properly selected because, in most metabolism studies with rats, majority of the radioactivity was excreted within 24 hrs (Hoechst Report No.: A33895).

After 24 hrs after dosing, approximately 21% and 24% of the administered radioactivity was excreted by males and females, respectively. Unmetabolized Hoe 039866 accounted for the majority of the excreted radioactivity. In urine, approximately 4% of the administered dose was excreted within 24 hrs. Other studies, in which two of the authors of this report were involved, indicated that approximately 80% of the administered radioactivity was excreted within the first 24 hrs (Hoechst Report Nos.: A33895, A33239, & A33975). An explanation should be offered in regards to this difference.

Two metabolites, Hoe 061517 and Hoe 086486 were found in urine, liver, and kidneys. Based upon these findings, a metabolism scheme was proposed (Figure 1, taken from page 30 of the report). In this scheme, Hoe 061517 was formed from oxidative desamination and subsequent decarboxylation. Hoe 061517 could further be dehydrated, hydroxylated, and finally dehydrated to form Hoe 086484.

The report presents useful information concerning the metabolites of the test article; however, the experimental procedures applied the pooling of the samples of all animals in each sex, and the results as presented in the report appeared to be derived from a single determination. They are similar to summary data which can not be verified by individual animal data or determinations. In addition, the number of test animals/sex (3/sex) was insufficient in this study. Therefore this study is classified as Supplementary.

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Page_____ is not included in this copy.

Pages 407 through 415 are not included.

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Reviewed by: Whang Phang, Ph.D. 8/18/88
Secondary Reviewer: Marcia van Gemert, Ph.D. 8/18/88
Section III, Tox. Branch (TS-769c)

DATA EVALUATION REPORT

006936

STUDY TYPE: Metabolism: Single oral Dose (Rat)

CHEMICAL: HOE 039866; monoammonium [2-amino-4-(hydroxymethyl-phosphinyl)butanoate]; Ignite®

EPA ACCESSION No: 403456-38 CASWELL No.: 580I

EPA ID No: 8340-EO/8340-EI EPA PROJECT No: 8-0146

SPONSOR: Hoechst Celanese Corp.

TESTING LABORATORY: Hoechst AG, 6230 Frankfurt AM Main 80
Federal Republic of Germany

CITATION: Wink, O. et al. (1986). Hoe 039866 ^{14}C-, Metabolism in male and female rats after a single oral administration 2 mg/kg body weight each. Hoechst AG, Germany; Report No.: A33895; Project No.: CM082/85 I. July 24, 1986. Submitted by Hoechst Celanese Corp., Sommerville, NJ. Aug 27, 1987.

SUMMARY: When 10 rats/sex were gavaged with a nominal dose of 2 mg/kg ^{14}C-Hoe 039866, greater than 80% of the applied radioactivity was excreted in the feces during the first 24 hrs. Unmetabolized Hoe 039866 practically accounted for all the excreted radioactivity. No other metabolite was detected in the urine or fece samples. The pattern of excretion was similar in male and female animals.

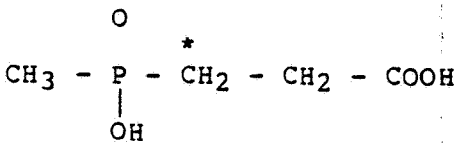
This study has many deficiencies which include (1) the feces, urine, or tissue samples from all animals in each sex were pooled and examined as a single sample instead of examining the samples on an individual animal basis, (2) the results appeared to be derived from single determinatons, and as such the data could only be considered as summary data which could not be verified, (3) the radioactivity in cage wash was never determined, and (4) the sacrifice date was never mentioned in the report. Therefore, this study is classified as Supplementary.

MATERIALS AND METHODS:

Test article: Unlabelled Hoe 039866 (Code No.: Hoe 039866 CH ZB 0001); purity 98.6%

Labelled Hoe 039866 (3,4-^{14}C-); Batch No.: 15044b
Specific radioactivity: 54.08 mCi/g; Radiochemical purity: 98%

Reference substance for a potential metabolite: Hoe 061517 or 3-methylphosphinico-propionic acid



Specific activity: 24.7 mCi/g; radiochemical purity: 98.7%

Preparation of the test article: 1.02 mg of labelled Hoe 039866 was mixed with 0.98 mg of non-labelled Hoe 039866 and dissolved in water. The final concentraton of the Hoe 039866 was 0.4239/ml and the specific radioactivity was 25.2 mCi/gm

The administered volumes were: 5.8 ml/kg (males)
6.7 ml/kg (females)

Test animals: Wistar rats [Hoe WISKF (SPF 71)] were obtained from Hoechst AG, Kastengrund. They were approximately 10 weeks (wks) old and weighed 145 -150 g for males and 190 - 200 g for females.

EXPERIMENTAL PROCEDURES:

Groups of 10 rats/sex were orally administered (by gavage) labelled Hoe 039866 at a nominal dose of 2 mg/kg, but the the actual doses were 2.46 mg/kg for males; 2.85 mg/kg, for females. Two rats/sex were used as controls. Samples of urine and feces were collected at 0 - 24, 24 -48, and 48 - 96 hours (hrs) post dosing. Samples of urine or feces from all animals of each sex were pooled. The report did not specify when the test animals were sacrificed. At sacrifice samples of liver, kidneys, fat, brain, spleen, and blood were collected. Like the samples of urine and feces, each organ or tissue sample of all animals from one sex were pooled for chemical analyses.

High performance liquid chromatography (HPLC) was used to identify possible metabolites, and the radioactivity was measured by liquid scintillation counting. The details of these analytical methods are excerpted from the report and presented in the Appendix.

RESULTS:

Excretion: The results of pooled samples of all animals in each sex are presented in Table I (taken from page 24 of the report). The data showed that 86.6% and 94.2% of the administered radio-

activity was excreted during the first 24 hrs for females and males, respectively. The majority of the radioactivity was excreted in the feces (80.5% and 88.2% of the administered radioactivity for females and males, respectively). The total recovery of the radioactivity in feces and urine was greater than 98% of the administered radioactivity for both males and females (Table I).

Radioactivity in organs and tissues:

The amounts of radioactivity in various tissues are presented in Table II (taken from page 25 of the report). Among the organs and tissues measured, liver and kidneys contained more radioactivity than other organs, but the quantities in these organs were less than 0.1% of the administered radioactivity.

Since the amount of radioactivity was rather low in various organs and tissues, the identification of possible metabolite was not conducted in these pooled sample.

Metabolites in urine and feces

Identification of possible metabolites was carried out with urine samples of 0 - 24 hrs and fece samples of 0 - 24 and 24 - 48 hrs. In the urine samples the potential metabolite, Hoe 061517, was below the detection limit. The results are presented in Table III (taken from page 26 of the report). The unmetabolized Hoe 039866 was found in both male and female urine in similar amounts (approximately 6% of the administered radioactivity).

In feces, the radioactivity was found in two fractions, an "extractable" and an "unextractable" fraction. In the fece samples of 0 - 24 hrs, unmetabolized Hoe 039866 accounted for all the radioactivity in the "extractable" fraction (71.1% and 75.9% of the administered radioactivity for females and males, respectively). In the "unextractable" fraction, the Hoe 039866 was 9.4% and 12.3%, for females and males, respectively (Table III). The level of potential metabolite, Hoe 061517, was below the detection level.

The radioactivity in 24 - 48 hrs fece samples was substantially less than that in the samples of 0 - 24 hrs. The unmetabolized Hoe 039866 accounted for all the radioactivity in the "extractable" feces fraction of both male and female rats (Table III).

From 0 - 96 hrs, unmetabolized Hoe 039866 accounted for 86.8% and 86.1% of the applied radioactivity in combined urine and "extractable" feces samples of female and male rats, respectively (Table IV, which is taken from page 27 of the report).

DISCUSSION:

Groups of rats (10/sex) were orally administered labelled Hoe 039866 at a nominal dose of 2 mg/kg, the majority of the administered radioactivity was excreted within 24 hrs. The major route of excretion was via feces. The unmetabolized Hoe 039866 essentially accounted for all the radioactivity in the feces and urine. The pattern of excretion for Hoe 039866 was similar for male and female rats under the current testing conditions.

Using the results from other animal studies, the results of which are not included in the report, the authors of the report proposed a metabolic pathway for Hoe 039866. The scheme as presented in the report is shown in Figure 1 (taken from Figure 7 and page 34 of the report). It should be noted that based upon the data presented in this report the proposed metaboic pathway could not be verified.

This study has many deficiencies which include (1) the feces, urine, or tissue samples from all animals in each sex were pooled and examined as a single sample instead of examining the samples on an individual animal basis, (2) the results appeared to be derived from a single determination, and as such the data could only be considered as summary data which could not be verified, (3) the radioactivity in cage wash was never determined, and (4) the sacrifice date was never mentioned in the report. Therefore, this study is classified as Supplementary.

RIN 5218-93 / TOX Review for Glufosinate Log #293

Page_____ is not included in this copy.

Pages 420 through 435 are not included.

The material not included contains the following type of information:

____ Identity of product inert ingredients.

____ Identity of product impurities.

____ Description of the product manufacturing process.

____ Description of quality control procedures.

____ Identity of the source of product ingredients.

____ Sales or other commercial/financial information.

____ A draft product label.

____ The product confidential statement of formula.

____ Information about a pending registration action.

✓ FIFRA registration data.

____ The document is a duplicate of page(s) _____.

____ The document is not responsive to the request.

The information not included is generally considered confidential by product registrants. If you have any questions, please contact the individual who prepared the response to your request.

Reviewed by: Whang Phang, Ph.D. 8/22/88
Secondary Reviewer: Marcia van Gemert, Ph.D.
Section III, Tox. Branch (TS-769c) 8/23/88

DATA EVALUATION REPORT

006936

STUDY TYPE: Metabolism: Single oral Dose (Rat)(30 mg/kg)

CHEMICAL: HOE 039866; monoammonium [2-amino-4-(hydroxymethyl-phosphinyl)butanoate]; Ignite®

EPA ACCESSION No: 403456-39 CASWELL No.: 580I

EPA ID No: 8340-EO/8340-EI EPA PROJECT No: 8-0146

SPONSOR: Hoechst Celanese Corp.

TESTING LABORATORY: Hoechst AG, 6230 Frankfurt AM Main 80
Federal Republic of Germany

CITATION: Wink, O. et al. (1986). Hoe 039866 ^{14}C-, Metabolism in male and female rats after a single oral administration of 30 mg/kg body weight each. Hoechst AG, Germany; Report No.: A33894; Project No.: CM083/85. May 8, 1986. Submitted by Hoechst Celanese Corp., Sommerville, NJ. Aug 27, 1987.

DISCUSSION AND SUMMARY:

This study was carried out by the same group of researchers as those of the study with Hoechst Report No.: A33895 and with similar experimental design. A copy of the experimental procedures was attached to show that the study was not well designed, and the study had the following deficiencies which precluded a proper evaluation:

(1) The feces, urine, or tissue samples from all animals in each sex were pooled and examined as a single sample instead of examining the samples on an individual animal basis.

(2) the results appeared to be derived from single determination and as such the data could only be considered as summary data which could not be verified,

(3) the radioactivity in the cage washes was never determined.

Based upon these observations, a detailed Data Evaluation Report will not be prepared for this study, and it is classified as Supplementary.

RIN 5218-93 / TOX Review for Glufosinate Log #293

Page_____ is not included in this copy.

Pages 437 through 443 are not included.

The material not included contains the following type of information:

____ Identity of product inert ingredients.

____ Identity of product impurities.

____ Description of the product manufacturing process.

____ Description of quality control procedures.

____ Identity of the source of product ingredients.

____ Sales or other commercial/financial information.

____ A draft product label.

____ The product confidential statement of formula.

____ Information about a pending registration action.

✓ FIFRA registration data.

____ The document is a duplicate of page(s) _____.

____ The document is not responsive to the request.

The information not included is generally considered confidential by product registrants. If you have any questions, please contact the individual who prepared the response to your request.

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

006936

MAY 5 1988

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Overview of Submitted Mutagenicity Studies on Ignite

FROM: Kerry L. Dearfield, Ph.D.
Geneticist
Scientific Mission Support Staff
Toxicology Branch
Hazard Evaluation Division (TS-769C)

TO: Whang Phang, Ph.D.
Section III
Toxicology Branch
Hazard Evaluation Division (TS-769C)

THRU: Reto Engler, Ph.D.
Chief
Scientific Mission Support Staff
Toxicology Branch
Hazard Evaluation Division (TS-769C)

Chemical: Ignite CAS# 77182-82-2 Caswell #580I

This reviewer has been requested to examine and summarize mutagenicity studies concerning Ignite that have been submitted to OPP. The following is a listing of these studies with their result and classification for acceptance:

Acceptable studies:

Salmonella assay: negative, Document #004403
E. coli reverse mutation assay: negative, Document #004403
Mouse lymphoma assay: negative, new DER
UDS/primary rat hepatocytes: negative, new DER

Unacceptable studies:

Salmonella assay: another assay from one above, negative, Document #004403
B. subtilis rec assay: negative, Document #004403, however, results from non-activated portion are adequate
Mouse micronucleus: negative, Documents #004403 & #004923
S. pombe forward mutation assay: negative, new DER

S. cerevisiae mitotic gene conversion: negative, new DER
Chromosome aberrations in cultured human lymphocytes: negative, new DER

The following will discuss these studies and then present an overall conclusion.

A. Salmonella assays

Two Salmonella assays were considered in Document #004403. In the acceptable study, Salmonella strains TA98, TA100, TA1535, TA1537 and TA1538 were exposed to test substance ± activation at concentrations up to 1000 ug/plate. There was virtual inhibition of growth noted in all strains at 1000 ug/plate. The results were negative for inducing an increased mutation frequency.

The second Salmonella assay was considered unacceptable. The same five strains were used and exposed to 0.5 ul/plate and negative results also obtained. While toxicity may be apparent at the highest concentration tested as the number of revertants dropped, the original reviewer considered this assay to not demonstrate toxicity (to upgrade this assay, the registrant should detail this aspect more fully). Other reasons for an unacceptable decision were actual concentrations of test material were not reported (only volumes provided) and the technical grade of the compound was not used.

B. E. coli reverse mutation assay (Document #004403)

An acceptable E. coli reverse mutation assay was performed with strain B/r WP2 hcr^- try^- at concentrations up to 1000 ug/plate. Virtual inhibition of growth was noted at the top concentration. The results were negative for inducing an increased mutation frequency.

C. Mouse lymphoma assay (new DER)

Test substance at concentrations up to 5000 ug/ml ± activation did not increase the mutation frequency after a 4 hour exposure. Top toxicities at 5000 ug/ml ranged from 28.3% to 73.6% relative growth (5000 ug/ml is a top recommended test concentration for this test).

D. Unscheduled DNA synthesis (UDS) in primary rat hepatocytes (new DER)

Test substance at concentrations up to 5240 ug/ml did not induce UDS after an 18-19 hour exposure. The top concentration produced 39.5% survival. The positive control was adequate.

E. B. subtilis rec assay (Document #004403)

Strains H17 (rec^+) and M45 (rec^-) were exposed to test

substance at concentrations up to 10,000 ug/plate under non-activated conditions only. There were no differences in growth between the two strains, indicating no detectable genotoxic effect. The original review states that this assay is acceptable, however, since activated conditions were not utilized, this assay should be considered unacceptable. However, the results without activation are adequate.

F. Mouse micronucleus assay (Documents #004403, #004928)

Five NMRI mice/sex/treatment group were exposed to 2 doses of 0, 8, 40 or 200 mg/kg of test substance by gavage 24 hours apart. They were sacrificed 6 hours after the second administration and bone marrow examined for micronuclei. In Document #004403, this assay was originally considered unacceptable as dosing did not appear justified (i.e. higher dosing may have been necessary). Document #004928 upgrades this assay to acceptable as a later submission provides the rationale for dose selection. Doses of 250 or 300 mg/kg/day produced clinical signs or were outright lethal to male and female mice. It was concluded that 200 mg/kg/day was a sufficient top dose.

However, this assay should remain as unacceptable. While the dosing may have been determined to be adequate, the protocol is not an acceptable one on a scientific basis. The sampling of bone marrow only six hours after the last dose does not allow adequate time for micronuclei to form from the last administration, and the sampling time does not allow for possible effects on cell cycle time to be taken into account. It is recommended that sampling of bone marrow should occur three times from at least 12 hours to up to 72 hours after the last dosing.

G. Schizosaccharomyces pombe forward mutation assay (new DER)

Concentrations up to 1000 ug/ml (based on a preliminary toxicity assay - no details given) were exposed for 4 hours to S. pombe. Survival was at 86% or greater in contrast to the recommended survival of at least 50% for a maximum dose. No mutagenic effect ± activation was observed with little concurrent toxicity. The original reviewer suggests that optimal conditions for expression were not provided; the stationary phase culture used was incubated at 32° C for 48 hours while recommended incubation is at 30° C for 36 hours to achieve optimum sensitivity.

H. Mitotic gene conversion in Saccharomyces cerevisiae D4 (new DER)

Concentrations of test substance of 1000, 2500, 5000 or 10,000 ug/ml ± activation did not increase conversion at the tryptophan or adenine loci. The cells were exposed for 4 hours and 32% survival was noted at the top concentration without activation and 68% survival with activation. However, the assay

was performed with stationary phase cultures, therefore the physiological state may have compromised the sensitivity of the assay. It is recommended that logarithmic phase cultures should be used to increase the sensitivity of the assay.

I. Chromosome aberration assay in cultured human lymphocytes (new DER)

Singly prepared cultures from one volunteer were incubated with 1, 10, 100 or 1000 ug/ml ± activation for 3 hours with test substance (after a 48 hour incubation from culture initiation in the presence of PHA). The cultures were washed and incubated for an additional 23 hours before colchicine was added for 3 more hours of incubation. A preliminary toxicity experiment showed that 55% of the control mitotic activity was noted at the top concentration without activation (activated conditions not tested). Over 90 metaphases/culture were scored and the positive controls appeared adequate. No increase in aberration frequency was observed.

This assay was considered unacceptable since only one culture per treatment group was established. It is recommended that two independent cultures be established per treatment group. Other problems with this assay include no toxicity established for cultures in the presence of activation (appears in this case the toxicity may be different and therefore different concentrations could have been tested). Also, the original reviewer suggests that the method of scoring slides may not be totally adequate. Aberrations were scored from projected film slides which may limit analysis to a single plane; the preferred method is to score slides directly from the microscope to examine all planes in the three dimensional preparation. Finally, slides were not apparently coded.

Overall Conclusions

Based on the weight-of-evidence from the submitted studies on Ignite, there does not appear to be a mutagenicity concern for Ignite at this time. However, the regulatory requirements have not been totally satisfied for the mutagenicity testing of Ignite. Acceptable results have satisfied the requirements for examining gene mutations (Salmonella, E. coli and mouse lymphoma assays) and other genotoxic effects (UDS). However, there are no acceptable studies to satisfy the requirement for structural aberrations. This is a data gap for regulatory purposes.

cc: M. van Gemert
Section Chief

006936

EPA: 68-02-4225
DYNAMAC No. 343-A
February 16, 1988

DATA EVALUATION RECORD

IGNITE

Mutagenicity--Forward Mutation in Schizosaccharomyces pombe Assay

STUDY IDENTIFICATION: Hirsch, I. E. and Milone, M. F. Study of the mutagenic activity in vitro of the compound HOE 039866 substance technical (code HOE 039866 OH ZC 95 0001) with Schizosaccharomyces pombe. (Unpublished study No. A29303 prepared by Instituto di Ricerche Biomediche, "Antoine Marxer," Ivrea, Italy, for Hoechst Celanese Corp., Somerville, NJ; dated June 19, 1984.) Accession No. 403456-15.

APPROVED BY:

Robert J. Weir, Ph.D.
Acting Department Manager
Dynamac Corporation

Signature: Robert J. Weir

Date: 2-16-88

1. CHEMICAL: HOE 039866; Ignite. 006936

2. TEST MATERIAL: HOE 039866 technical, code No. HOE 039866 OH ZC 95 0001 was described as a white crystalline powder with a purity of 95.3%.

3. STUDY/ACTION TYPE: Mutagenicity--Forward mutation in Schizosaccharomyces pombe assay.

4. STUDY IDENTIFICATION: Hirsch, I. E. and Milone, M. F. Study of the mutagenic activity in vitro of the compound HOE 039866 substance technical (code HOE 039866 OH ZC 95 0001) with Schizosaccharomyces pombe. (Unpublished study No. A29303 prepared by Instituto di Ricerche Biomediche, "Antoine Marxer," Ivrea, Italy, for Hoechst Celanese Corp., Somerville, NJ; dated June 19, 1984.) Accession No. 403456-15.

5. REVIEWED BY:

Nancy E. McCarroll, B.S.
Principal Reviewer
Dynamac Corporation

Signature: Nancy McCarroll
Date: 2-16-88

Brenda Worthy, M.T.
Independent Reviewer
Dynamac Corporation

Signature: Brenda Worthy
Date: 2-16-88

6. APPROVED BY:

I. Cecil Felkner, Ph.D.
Genetic Toxicology
Technical Quality Control
Dynamac Corporation

Signature: I Cecil Felkner
Date: 2-16-88

Whang Phang, Ph.D.
EPA Reviewer

Signature:
Date: 3/15/88

Marcia Van Gemert, Ph.D.
EPA Section Head

Signature:
Date: 3/16/88

7. CONCLUSIONS:

A. Under the conditions of this assay, four doses of HOE 039866 ranging from 125 to 1000 µg/mL, both in the presence and absence of S9 activation, did not induce a mutagenic effect in Schizosaccharomyces pombe; however, the study cannot be assessed for the following reasons:

1. Optimal conditions for expression of mutation were not provided. The stationary phase culture was incubated at 32°C for 48 hours; this is in contrast to the recommended incubation at 30°C for 36 hours required to achieve optimum sensitivity.[1]

2. No indication of variability in mutant and survivor plate counts was reported.

B. The study is unacceptable.

8. RECOMMENDATIONS: It is recommended that the repeat assay be performed in a manner consistent with established procedures.[2] Given the biological similarities between S. pombe and Saccharomyces cerevisiae, it is further suggested, in accordance with Zimmerman's recommendations for S. cerevisiae mutation studies,[3] that overall assay sensitivity may be improved if exponentially grown S. pombe cells are used. In addition, preliminary cytotoxicity data and the rationale for selecting the dose levels should be included in the report.

Items 9-10--see footnote 4.

11. MATERIALS AND METHODS (PROTOCOLS):

A. Materials and Methods: (See Appendix A for details.)

1. Test Material: HOE 039866 technical was described as a white crystalline powder; the purity was listed as 95.3%. The test

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[1]Loprieno, N. Screening of coded carcinogenic/noncarcinogenic chemicals by a forward-mutation system with the yeast Schizosaccharomyces pombe, in: Evaluation of Short-Term Tests for Carcinogens, ed. de Serres, F. J. and Ashby, J., 1981, Elsevier/North Holland Biomedical Press, NY, pp. 424-433.

[2]Ibid.

[3]Zimmerman, F. K. Procedures used in the induction of mitotic recombination and mutation in the yeast Saccharomyces cerevisiae, in: Handbook of Mutagenicity Test Procedures, ed. Kilbey, B. J., Legator, M., Nichols, W., and Ramel, C., 1979. Elsevier/North Holland Biomedical Press, NY, pp. 119-134.

[4]Only items appropriate to this DER have been included.

material was reported to be stable under assay conditions and was dissolved in sterile, deionized water (DH_2O).

2. S. pombe double mutant haploid P_1 strain (SP ade 6-60/rad 10-198, h^-) was obtained from "Laboratorio di Mutagenesi e Differenziamento," Pisa, Italy, and was maintained as a permanent stock on silica gel. Cultures for the assay were grown from this stock in 100 mL of liquid yeast media (3% glucose, 0.5% yeast extract, and 0.0075% adenine sulfate), shaken for 48 hours at 32°C, washed, and resuspended in physiological saline.

3. The S9 fraction used for metabolic activation was prepared from the livers of Aroclor-1254-induced male Sprague-Dawley rats. Each batch was assayed for protein content and mutagenic activity in Salmonella typhimurium TA1538, TA98, and TA100 using the promutagen, 2-aminofluorene.

4. Cytotoxicity Test: Dose levels were chosen on the basis of a preliminary toxicity test; the procedure was not described.

5. Mutagenicity Test: The maximum dose used for the mutation assay was selected to achieve a survival rate of at least 50%. Four doses of the test material, the negative control (DH_2O), or the positive controls (84.5 µg/mL methyl methanesulfonate (MMS) for the nonactivated and 375 µg/mL dimethylnitrosamine (DMN) for the S9-activated exposure) were added in 0.1-mL volumes to tubes containing 1 mL of 5.5×10^8 cells and 2.9 mL phosphate buffer, pH 7.4. Tubes used for the S9-activated series contained 1.9 mL phosphate buffer and 1 mL of the S9 mix. Reaction mixtures were incubated at 35°C with shaking for 4 hours, diluted, and plated for survival (4 plates) and mutation (14 plates). Plates were incubated at 32°C for 4 days. The numbers of white (mutant) and red (wild type) colonies were counted; relative survival and mutation frequencies (MF) were determined.

6. Evaluation Criteria: No criteria for a positive response, the validity of the assay, or the biological significance of the findings were presented.

7. The data were analyzed by the Chi-square test at $p < 0.05$, < 0.01, and < 0.001.

B. Protocol: A protocol was not provided.

12. REPORTED RESULTS:

A. Cytotoxicity Assay: No details were given for the preliminary range-finding experiment.

B. Mutation Assay: Based on the findings of the unreported cytotoxicity assay, the doses selected for the mutagenicity assay both with and without S9 activation were 125, 250, 500, and

1000 µg/mL. Without activation, these doses resulted in a survival range of 86% at the high dose to 100% at doses ≥500 µg/mL; with activation, survival was ≥91% at all doses. Under both conditions of activation, no significant increase in mutation of S. pombe was observed. By contrast, the nonactivated and S9-activated controls, MMS and DMN, induced significant ($p < 0.001$) increases in the MF.

Representative results are presented in Table 1.

13. STUDY AUTHORS' CONCLUSIONS/QUALITY ASSURANCE MEASURES:

A. The authors stated "Up to the concentration of 1000 µg/mL the test article HOE 039866-substance technical did not induce significant increases in gene mutation of Schizosaccharomyces pombe in vitro either in the presence or in the absence of hepatic microsomal enzymes."

B. A quality assurance study was signed and dated April 7, 1984.

14. REVIEWERS' DISCUSSION AND INTERPRETATION OF STUDY RESULTS:

We assess that HOE 039866 was tested up to a level that caused marginal cytotoxicity with no evidence of a mutagenic effect. However, the working stock suspension of S. pombe was incubated for a longer period (48 hours) and at a higher temperature (32°C) than is currently recommended by Loprieno,[5] who incubates working stock suspensions at 30°C for 36 hours. Therefore, the sensitivity of the cells to detect weak mutagenic activity was questionable. Although it may not be pertinent to S. pombe, Sharp and Perry,[6] in agreement with Zimmerman,[7] have demonstrated that exponential Saccharomyces cerevisiae cells are more responsive to certain mutagens (safrole, auramine, diethylstilbestrol, and n-nitrosomorpholine) than are stationary phase cultures. We are not aware of any studies exploring this possibility in S. pombe; however, the similarities between the two yeast cells suggest to us that increased sensitivity could be achieved if actively growing cultures are used. The ability of the test system to detect weak activity induced by unknown compounds has not been established for test material structurally unrelated to the positive controls used in this study. Additionally, the presentation of mean values without some indication of the variability (standard deviation) is not an acceptable practice.

Item 15-see footnote 4.

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[5] Loprieno, pp. 424-432.

[6] Sharp, D. C. and Perry, J. M. Induction of mitotic gene conversion by 41 coded compounds in yeast strain JD1, in: Evaluation of Short-Term Tests for Carcinogens. Elsevier/North Holland Biomedical Press, NY, pp. 491-502.

[7] Zimmerman, pp. 119-134.

TABLE 1. Representative Results of the S. pombe Forward Mutation Assay with HOE 039866

Substance	S9 Activation	Dose (µg/mL)	% Survival[a]	No. of Mutant Colonies[b]	Total Population[b] x 10^4	Mutation Frequency x 10^4
Solvent Control						
Deionized water	-		100	9	5.7	1.57
	+		100	8	6.1	1.30
Positive Control						
Methyl methanesulfonate	-	84.5	40	47	2.3	20.56*
Dimethylnitrosamine	+	375.0	59	21	3.6	5.78*
Test Material						
HOE 039866[d]	-	1000	86	10	4.9	2.03
	+	1000	91	5	5.6	0.90

[a] % Survival = $\frac{\text{Total Population of Test Dose}}{\text{Total Population of Solvent Control}}$ x 100.

[b] Average of 14 replicates for mutant counts; four replicates for total population.

[c] Mutation Frequency = $\frac{\text{No. of Mutant Colonies}}{\text{Total Population}}$.

[d] Highest dose; mutation frequencies for 125, 250, and 500 µg/mL were not significantly different from the solvent control.

*Significant increase in mutation frequency ($p < 0.001$) by Chi-square test.

16. CBI APPENDIX: Appendix A, Materials and Methods, CBI, pp. 5-10. 000936

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APPENDIX A

Materials and Methods

RIN 5218-93 / Tox Review for Glufosinate Log #293

Page_____ is not included in this copy.

Pages 456 through 461 are not included.

The material not included contains the following type of information:

____ Identity of product inert ingredients.

____ Identity of product impurities.

____ Description of the product manufacturing process.

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__✓__ FIFRA registration data.

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The information not included is generally considered confidential by product registrants. If you have any questions, please contact the individual who prepared the response to your request.

006936

EPA: 68-02-4225
DYNAMAC No. 343-B
February 16, 1988

006936

DATA EVALUATION RECORD

IGNITE

Mutagenicity--Rat Primary Hepatocyte Unscheduled DNA Synthesis Assay

STUDY IDENTIFICATION: Cifone, M. A., and Myhr, B. C. Evaluation of HOE 039866-substance technical in the rat primary hepatocyte unscheduled DNA synthesis assay. (Unpublished study No. 20991 prepared by Litton Bionetics, Inc., Kensington, MD, for Hoechst Celanese Corp., Somerville, NJ; dated November 1984.) MRID No. 403456-14.

APPROVED BY:

Robert J. Weir, Ph.D.
Acting Department Manager
Dynamac Corporation

Signature: Ira Cecil Dillingham

Date: 2-16-88

2. CHEMICAL: Ignite; HOE 039866.

2. TEST MATERIAL: HOE 039866-substance technical, code No. HOE 039866 OH ZC95 0001, was a white powder with a purity of 95.3 percent.

3. STUDY/ACTION TYPE: Mutagenicity--Rat primary hepatocyte unscheduled DNA synthesis assay.

4. STUDY IDENTIFICATION: Cifone, M. A., and Myhr, B. C. Evaluation of HOE 039866-substance technical in the rat primary hepatocyte unscheduled DNA synthesis assay. (Unpublished study No. 20991 prepared by Litton Bionetics, Inc., Kensington, MD, for Hoechst Celanese Corp., Somerville, NJ; dated November 1984.) MRID No. 403456-14.

5. REVIEWED BY:

Nancy E. McCarroll, B.S.
Principal Reviewer
Dynamac Corporation

Signature: Nancy E. McCarroll
Date: 2-16-88

Brenda Worthy, M.T.
Independent Reviewer
Dynamac Corporation

Signature: Brenda Worthy
Date: 2-16-88

6. APPROVED BY:

I. Cecil Felkner, Ph.D.
Genetic Toxicology
Technical Quality Control
Dynamac Corporation

Signature: I. Cecil Felkner
Date: 2-16-88

Whang Phang, Ph.D.
EPA Reviewer

Signature: Whang Phang
Date: 3/16/88

Marcia Van Gemert, Ph.D.
EPA Section Head

Signature: M. van Gemert
Date: 3/16/88

7. CONCLUSIONS:

A. Under the conditions of the assay, eight doses of HOE 039866-substance technical, ranging from 26.2 to 5240 µg/mL, did not induce appreciable changes in the pattern of nuclear labeling of rat hepatocytes. These doses resulted in a cell survival range of 39.5 to 108.1 percent. HOE 039866-substance technical is, therefore, considered cytotoxic but not genotoxic in the primary rat hepatocyte unscheduled DNA synthesis (UDS) assay.

B. The study is acceptable.

Items 8 through 10--see footnote 1.

11. MATERIALS AND METHODS (PROTOCOLS):

A. Materials and Methods: (See Appendix A for details.)

1. Test Material: HOE 039866-substance technical was described as a white powder with a purity of 95.3 percent. A stock solution of the test material was prepared in deionized water to contain 524 mg/mL; subsequent dilutions were made in Williams' Medium E (WME) to yield 15 concentrations ranging from 0.1 to 5240 µg/mL. The stock solution and serial dilutions were prepared immediately prior to use under reduced lighting.

2. Indicator Cells: Hepatocytes were obtained from a male Fischer 344 rat (150-300 g) quarantined for a minimum of 5 days. This animal was identified by cage card and was fed Purina Certified Chow (formula 5002) and water ad libitum.

3. Cell Preparation:

a. Perfusion Technique: The liver was perfused with Hanks' balanced salts containing 0.5 mM EGTA and HEPES pH 7.0 buffer for 4 minutes and with WME containing 50-100 units/mL collagenase for 10 minutes. The liver was excised and mechanically dispersed in WME and collagenase to release the hepatocytes.

b. Hepatocyte Harvest/Culture Preparation: Recovered cells were centrifuged, resuspended in WME, counted, and aliquoted (0.5×10^6 cells, 3 mL WME) onto plastic coverslips. The cultures were placed in a humidified, 37°C, 5 percent CO_2 incubator for a 1.5- to 2-hour attachment period. Unattached cells were removed; viable cells were refed and established as monolayer cultures.

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[1] Only items appropriate to this DER are included.

4. Dose Selection: Initially, 15 concentrations from approximately two-fold dilutions of the test material were assayed. Viability was determined 20 to 24 hours after initiation of treatment by trypan blue exclusion. Based on these findings, at least six doses were chosen to analyze nuclear labeling; these included the highest dose that resulted in a sufficient number of survivors with intact morphologies and proceeded to the successively lower doses.

5. UDS Assay:

   a. Treatment: Five replicate, monolayer cultures were exposed to the selected doses of the test material and negative or positive controls (2-acetylaminofluorene 2-AAF, 0.1 µg/mL for 18 to 19 hours in WME containing 1 µCi/mL [^{3}H]thymidine. Treated monolayers were washed twice with WME and two of the five replicates from each treatment group were used to determine cytotoxicity. These cultures were refed, reincubated, and monitored for cytotoxicity at 20 to 24 hours posttreatment.

   b. UDS Slide Preparation: The remaining cultures (three replicate coverslips/treatment group) were washed with media containing 1 mM thymidine. Treated hepatocytes attached to coverslips were exposed to 1 percent sodium citrate for 8 to 10 minutes, fixed in acetic acid:ethanol (1:3), dried, and mounted.

   c. Preparation of Autoradiographs/Grain Development: Slides were coated with Kodak NTB2 emulsion, dried for 7 to 10 days at 4°C in light-tight dessicated boxes, developed in Kodak D-19, fixed, stained with Williams' modified hematoxylin and eosin, coded, and counted.

   d. Grain Counting: The nuclear grains of 150 morphologically normal cells for each test dose and negative and positive controls were counted microscopically. Net nuclear grain counts were determined by subtracting the nuclear grain counts of each cell from the average cytoplasmic grain count of three nuclear-sized areas adjacent to each nucleus.

6. Evaluation Criteria:

   a. Assay Validity: The assay was considered valid if a) pretreatment cell viability was >50%, b) net nuclear counts for the negative control did not exceed an average of 2 grains per nucleus; or no more than 10% of the control cells had ≥6 grains; or no more than 1% had ≥20 grains; and c) the sensitivity of the assay to detect UDS was demonstrated by the positive control.

b. Positive Response: The assay was considered positive if a) an increase in the mean nuclear grain count was ≥6 grains/nucleus over the negative control value, or b) the percent of nuclei with ≥6 grains exceeded 10 percent of the negative control population, or c) the percent of nuclei with ≥20 grains was ≥2 percent of the examined population.

8. Protocol: See Appendix B.

12. REPORTED RESULTS:

UDS Assay: Cytotoxicity and the UDS assay were performed in parallel. Fifteen test concentrations ranging from 0.1 to 5240 µg/mL were used. The authors stated that all doses appeared to be soluble and did not alter the pH of the culture medium. Cell survival ranged from 39.5% at the high dose to ~100% at doses ≤262 µg/mL. Survival was not determined for levels below 52.4 µg/mL. Based on these observations, eight treatments (ranging from 26.2 to 5240 µg/mL) were selected to analyze nuclear labeling. Results showed no appreciable increase in nuclear grain counts of cells exposed to graded doses of the test material compared to the solvent control. By contrast, the positive control (0.1 µg/mL 2-AAF) induced a marked increase in UDS.

Representative results are presented in Table 1.

13. STUDY AUTHORS' CONCLUSIONS/QUALITY ASSURANCE MEASURES:

A. The authors concluded that "the test material HOE-039866-substance technical, did not induce significant changes in the nuclear labeling of primary rat hepatocytes" and "therefore, the test material was evaluated as inactive in the Primary Rat Hepatocyte UDS Assay."

B. A quality assurance statement was signed and dated November 9, 1984.

14. REVIEWERS' DISCUSSION AND INTERPRETATION OF STUDY RESULTS:

We assess that the study was conducted properly and that the authors' interpretation of the data was correct.

None of the doses induced an appreciable increase in UDS grains/nuclei, and a satisfactory reduction in the viability of hepatocytes (60.5% at 5240 µg/mL) was demonstrated. These results show that the negative UDS response was not due to the inability of the test material to penetrate the cell target site.

TABLE 1. Representative Results of the Unscheduled DNA Synthesis Rat Hepatocyte Assay with HOE 039866-Substance Technical

Treatment	Dose	Cells Scored	Percent Survival[a] (22 Hours)	Average Net Nuclear Grain Count	Average Percent Nuclei w/ ≥6 Grains	Average Percent Nuclei w/ >20 Grains
Negative Control						
Williams' Medium	-	150	100	0.63	0.0	0.0
Positive Control						
2-Acetylaminofluorene	0.1 µg/mL[c]	150	79.0	14.28[b]	80.7[b]	26.7[b]
Test Material						
HOE 039866	5240 µg/mL[c]	150	39.5	0.56	0.7	0.0

[a] Percent survival = $\frac{\text{No. of viable cells/unit area test dose}}{\text{No. of viable cells/unit area solvent control}} \times 100.$

[b] Fulfills reporting laboratory's criteria for positive effect.

[c] Highest assayed dose; nuclear grain counts for lower doses (26.2, 52.4, 105, 262, 524, 1050, and 2620) were comparable to the negative control.

All criteria established by the study authors to judge assay validity were satisfied; we conclude, therefore, that HOE 039866 was assayed to a cytotoxic level and that there was no indication of genotoxicity in this acceptable study.

Item 15--see footnote 1.

16. CBI APPENDIX: Appendix A, Materials and Methods, CBI pp. 0005-0006; Appendix B, Protocol, CBI pp. 0010-0016.

APPENDIX A

Materials and Methods

RIN 5218-93 / Tox Review for Glufosinate Log #293

Page_____ is not included in this copy.

Pages 470 through 479 are not included.

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____ Identity of product impurities.

____ Description of the product manufacturing process.

____ Description of quality control procedures.

____ Identity of the source of product ingredients.

____ Sales or other commercial/financial information.

____ A draft product label.

____ The product confidential statement of formula.

____ Information about a pending registration action.

✓ FIFRA registration data.

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006936

CONFIDENTIAL BUSINESS INFORMATION
DOES NOT CONTAIN
NATIONAL SECURITY INFORMATION (EO 12065)

EPA: 68-02-4225
DYNAMAC No. 343-D
February 16, 1988

DATA EVALUATION RECORD

IGNITE

Mutagenicity--Mitotic Gene Conversion in Saccharomyces cerevisiae D_4

STUDY IDENTIFICATION: Mellano, D. and Berruto, G. Study of the mutagenic activity of the compound HOE 039866-substance technical (code HOE 039866 OH ZC 95 0001) with Saccharomyces cerevisiae. (Unpublished study No. A29302 prepared by Istituto Di Ricerche Biomediche, "Antoine Marxer," Ivrea, Italy, for Hoechst Celanese Corp., Somerville, NJ; dated June 19, 1984.) MRID No. 403456-17.

APPROVED BY:

Robert J. Weir, Ph.D.
Acting Department Manager
Dynamac Corporation

Signature: Robert J. Weir for

Date: 2-16-88

480

1. CHEMICAL: HOE 039866; Ignite.

2. TEST MATERIAL: HOE 039866 technical, code No. HOE 039866 OH ZC 95 0001, was described as a white crystalline powder with a purity of 95.3%.

3. STUDY/ACTION TYPE: Mutagenicity--Mitotic gene conversion in Saccharomyces cerevisiae D_4.

4. STUDY IDENTIFICATION: Mellano, D. and Berruto, G. Study of the mutagenic activity of the compound HOE 039866-substance technical (code HOE 039866 OH ZC 95 0001) with Saccharomyces cerevisiae. (Unpublished study No. A29302 prepared by Istituto Di Ricerche Biomediche, "Antoine Marxer," Ivrea, Italy, for Hoechst Celanese Corp., Somerville, NJ; dated June 19, 1984.) MRID No. 403456-17.

5. REVIEWED BY:

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Date: 2-16-88

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6. APPROVED BY:

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Signature: Whang Phang
Date: 3/15/88

Marcia Van Gemert, Ph.D.
EPA Section Head

Signature: Marcia Van Gemert
Date: 3.16.88

7. CONCLUSIONS:

A. Under the conditions of the Saccharomyces cerevisiae D_4 mitotic gene conversion assay, HOE 039866 at concentrations of 1000, 2500, 5000, or 10,000 µg/mL, either in the presence or absence of S9 activation, did not cause an appreciable increase in tryptophan or adenine convertants. However, the assay was performed with stationary-phase cultures; hence, this physiological state may have compromised the sensitivity of the assay.

B. The assay is unacceptable.

8. RECOMMENDATIONS: It is recommended that the assay be repeated using logarithmic phase cells.

Items 9 and 10--see footnote 1.

11. MATERIALS AND METHODS (PROTOCOLS):

A. Materials and Methods: (See Appendix A for details.)

1. Test Material: HOE 039866 technical was described as a white crystalline powder; the purity was listed as 95.3%. The test material was reported to be stable under assay conditions and was dissolved in sterile deionized water (DH_2O).

2. Microbial Strain:

a. Strain Description/Source: S. cerevisiae diploid strain D_4 was obtained from Laboratorio di Mutagenesi e Differenziamento, Pisa, Italy, and was maintained in permanent silica gel stocks.

b. Stock Culture Preparation/Maintenance: Stock cultures of S. cerevisiae were generated from silica gel stocks and prescreened for low gene conversion frequency background. Silica granules were inoculated into 100 mL of liquid growth media (2% glucose, 1% yeast extract, 2% peptone, 0.004% adenine sulphate, and 0.004% tryptophan) and shaken for 16 hours at 32°C.

Following incubation, 0.05 mL volumes from a 10^{-5} dilution of the preculture(s) were inoculated into 10 replicate tubes containing 10 mL of fresh growth medium and incubated for 48 hours in a 32°C, shaking water bath.

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[1]Only items appropriate to this DER have been included.

The viable cell population was determined by plating a 10^{-5} dilution of the resulting culture(s) onto complete medium (2% glucose, 1% yeast extract, 2% peptone, and 1.5% agar). The number of background convertants was assessed by plating a 10^{-1} dilution onto selective medium (2.22% MM82, Biolife, and 1.5% agar supplemented with either 0.004% adenine sulphate or tryptophan). After 3 to 4 days of incubation at 32°C, the ratio of total colonies to adenine and tryptophan convertants was determined. The culture yielding the lowest convertant frequency (CF) for both genes was selected as the stock culture, and it was stored at 4°C until use in the assay.

c. Cell Preparation for Gene Conversion Assay: A 0.3-mL aliquot of the refrigerated stock suspension was added to 100 mL of liquid growth medium, incubated for 16 hours in a shaking, 32°C water bath, centrifuged, and resuspended in saline. Cell density was determined with a hemocytometer, and the suspension was adjusted to contain 5×10^8 cells/mL.

3. The S9 fraction used for metabolic activation was prepared from the livers of Aroclor 1254-induced male Sprague-Dawley rats. Each S9 batch was assayed for its protein content and mutagenic activity in Salmonella typhimurium TA1538, TA98, and TA100 using the promutagen 2-aminofluorene.

4. Cytotoxicity Test: Dose levels were chosen on the basis of a preliminary cytotoxicity test; the procedure was not described.

5. Mutagenicity Test: The maximum dose was chosen on the basis of achieving a survival rate of at least 30%. One milliliter of the four selected test doses or the negative control (DH_2O), was added to tubes containing 1 mL of 5×10^8 cells and 2.0 mL phosphate buffer, pH 7.4. Tubes used for metabolic activation contained 1.0 mL S9 mix. The positive controls were 84.5 µg/mL methyl methanesulfonate (MMS) for the nonactivated exposure and 259 µg/mL cyclophosphamide (CP) for the S9-activated exposures. Reaction mixtures were incubated at 35°C with shaking for 4 hours, diluted as described in Section II.A.2.b, and plated for viability (four plates) and convertants (four selective medium plates containing adenine sulphate and four selective medium plates containing tryptophan). After 4 days of incubation at 32°C, the number of survivors, tryptophan convertants, and adenine convertants were counted; relative survival and CFs were determined.

6. Statistical Methods: The data were analyzed by the Chi-square test at $p < 0.05$, < 0.01, and < 0.001.

7. Evaluation Criteria: No criteria for a positive response, the validity of the assay, or the biological significance of the findings were presented.

B. Protocol: A protocol was not provided.

12. REPORTED RESULTS:

A. Cytotoxicity Assay: No details were reported for the preliminary range-finding experiments.

B. Mitotic Gene Conversion Assay: Based on the findings of the unreported cytotoxicity assay, the doses selected for the S9-activated and nonactivated mitotic gene conversion assay were 1000, 2500, 5000, and 10,000 µg/mL. Of the yeast cells plated, 32% survived treatment with the nonactivated test material at 10,000 µg/mL. At 5000 µg/mL, 78% of the cells survived and 100% survival was reported for the two remaining lower doses. No appreciable increase in mutation at either the trp5 or ade2 loci resulted from exposure to the four nonactivated doses, and no significant increase in CF was observed.

Survival for yeast cells exposed to the high dose was 68% in the presence of S9 activation; survival was ≥90% at the remaining lower doses. Tryptophan and adenine convertants in dosed cultures were comparable to the solvent control, and no statistically significant increases in CFs were calculated.

Both the nonactivated (MMS) and the S9-activated (CP) positive controls induced significant increases in CFs at both loci. Representative results from the nonactivated and S9-activated mitotic gene conversion assay are presented in Table 1.

13. STUDY AUTHORS' CONCLUSIONS/QUALITY ASSURANCE MEASURES:

A. The authors concluded that "The results of this experiment indicate that the test article HOE 039866 - SUBSTANCE TECHNICAL at the concentration of up to 10,000 µg/mL did not induce significant increases in gene conversion in the Saccharomyces cerevisiae strain 'in vitro' either in the presence or in the absence of metabolic activation."

B. A quality assurance statement was signed and dated June 21, 1984.

14. REVIEWERS' DISCUSSION AND INTERPRETATION OF STUDY RESULTS:

A. We assess that the authors interpreted the data correctly. However, the assay was performed with working-stock suspensions of stationary-phase cultures; cultures were 16 hours old prior to preparation for the assay. To improve assay sensitivity,

TABLE I. Representative Results of the S. cerevisiae D_4 Mitotic Gene Conversion Assay with HOE 039866

Substance	S9 Activation	Dose (µg/mL)	Total Survivors x10^6	Percent Survival[a]	Convertants of S. cerevisiae[b] (x10^7) Trp$^+$	Ade$^+$	Convertant Frequency[c] (x10^{-5}) Trp$^+$	Ade$^+$
Negative Control								
Deionized water	-	—	9.1	100	20.5	20.5	2.27	2.27
	+	—	8.7	100	21.3	22.8	2.63	2.46
Positive Control								
Methyl methanesulfonate	-	84.5	9.5	104	70.3	63.8	6.69**	7.38**
Cyclophosphamide	+	259.0	9.8	113	58.8	53.8	5.50*	6.01*
Test Material								
HOE 039866	-	10,000[d]	2.9	32	5.8	4.8	1.62	1.97
	+		5.9	68	13.8	16.3	2.77	2.34

[a] Average of quadruplicate plates.

[b] Percent Survival = $\frac{\text{Total survivors with test dose}}{\text{Total survivors with solvent control}} \times 100.$

[c] Convertant Frequency = $\frac{\text{Total number of convertants (tryptophan or adenine)}}{\text{Total number of survivors}}$.

[d] Highest dose assayed; values for lower doses (1000, 2500, and 5000 µg/mL) were comparable to the negative control results.

*Significantly higher than control value ($p < 0.01$) by X^2 test.

**Significantly higher than control value ($p < 0.001$) by X^2 test.

Zimmermann et al.[2] have recommended the use of actively growing cells (i.e. cells that are grown approximately five hours prior to use). Although a different S. cerevisiae strain was used, Sharp and Parry,[3] in agreement with Zimmermann et al.,[4] have demonstrated that positive results with the known carcinogens/mutagens, safrole, auramine, diethylstilbestrol, and n-nitrosomorpholine, were only achieved when the treatment was administered to logarithmic phase cells.

Although the authors demonstrated the sensitivity of S. cerevisiae D_4 for detecting the mutagenic effect of the positive controls (84.5 µg/mL MMS/-S9 and 259 µg/mL CP/+S9), the use of stationary-phase cultures compromised the overall sensitivity of the test system. Unless the authors can provide information indicating that HOE 039866 is structurally related to the positive control mutagens, the responsiveness of the stationary-phase cells to weakly recombinogenic agents was not established.

Item 15--see footnote 1.

16. CBI APPENDIX: Appendix A, Materials and Methods, CBI pp. 0006-0012.

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[2]Zimmermann, F. K., von Borstel, R. C., von Halle, E. S., Parry, J. M., Siebert, D., Zetterberg, G., Barale, R., and Loprieno, N. Testing of chemicals for genetic activity with Saccharomyces cerevisiae: A Report of the U.S. Environmental Protection Agency Gene-Tox Program. Mutat. Res. 133(1984): 199-244.

[3]Sharp, D. C. and Parry, J. M. Introduction of mitotic gene conversion by 41 coded compounds in yeast strain JDI, in: Evaluation of Short-Term Tests for Carcinogens (New York, NY: Elsevier/North Holland, Vol. 1, 1981), pp. 491-501.

[4]Zimmermann et al., pp. 199-244.

APPENDIX A

Materials and Methods

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006936

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EPA: 68-02-4225
DYNAMAC No. 343-C
February 12, 1988

033036

DATA EVALUATION RECORD

HOE 039866 (IGNITE)

Mutagenicity--Mouse Lymphoma Forward Mutation Assay

STUDY IDENTIFICATION: Cifone, M. A. and Myhr, B. C. Mutagenicity evaluation of HOE 039866 substance technical in the mouse lymphoma forward mutation assay. (Unpublished study No. A30380 prepared by Litton Bionetics, Inc., Kensington, MD, for Hoechst Celanese Corp., Somerville, NJ; dated January 1985.) Accession No. 403456-16.

APPROVED BY:

Robert J. Weir, Ph.D.
Acting Department Manager
Dynamac Corporation

Signature: [signature]

Date: 2-16-89

1. CHEMICAL: HOE 039866; Ignite.

003936

2. TEST MATERIAL: HOE 039866 substance technical, code No. HOE 039866 OHZC95001, was described as a fine white powder with a purity of 95.3%.

3. STUDY/ACTION TYPE: Mutagenicity--mouse lymphoma forward mutation assay.

4. STUDY IDENTIFICATION: Cifone, M. A. and Myhr, B. C. Mutagenicity evaluation of HOE 039866 substance technical in the mouse lymphoma forward mutation assay. (Unpublished study No. A30380 prepared by Litton Bionetics, Inc., Kensington, MD. for Hoechst Celanese Corp., Somerville, NJ; dated January 1985.) Accession No. 403456-16.

5. REVIEWED BY:

Brenda Worthy, M.T.
Principal Reviewer
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Signature: Brenda Worthy
Date: 2-16-88

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I. Cecil Felkner, Ph.D.
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EPA Reviewer

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Marcia Van Gemert, Ph.D.
EPA Section Head

Signature: Marcia Van Gemert
Date: 3/16/88

7. CONCLUSIONS: 003936

A. Under the conditions of the mouse lymphoma L5178Y forward mutation assay, HOE 039866 substance technical, tested at seven nonactivated doses of 50 to 5000 µg/mL or at six S9-activated doses of 300 to 5000 µg/mL, did not increase the mutation frequency (MF) at the thymidine kinase (TK) locus. The solvent controls gave acceptable values and the positive controls ethylmethanesulfonate (EMS; nonactivated) and 3-methylcholanthrene (3-MCA; S9 activated) provided evidence that the assay had adequate sensitivity for detecting mutagenicity.

B. The study is acceptable.

Items 8 through 10--see footnote 1.

11. MATERIALS AND METHODS (PROTOCOLS):

A. Materials and Methods: (See Appendix A for details.)

1. Test Materials: HOE 039866 substance technical was described as a fine white powder with a purity of 95.3%. The test material was dissolved in deionized water, the solvent control.

2. Indicator Cells: The mouse lymphoma cell line L5178Y ($TK^{+/-}$) was derived from the 3.7.2 clone of Fischer L5178Y cells of Dr. Donald Clive, Burroughs Wellcome Company. Stock cultures were maintained in liquid nitrogen. Cultures were periodically checked for mycoplasma contamination and exposed to methotrexate to maintain a low background frequency of trifluorothymidine (TFT)-resistant cells.

3. S9 Fraction: The S9 fraction was commercially prepared by Litton Bionetics and was derived from the livers of adult male rats (strain not specified) induced with Aroclor 1254. The S9 mix contained nicotinamide adenine dinucleotide phosphate, isocitrate, and S9 fraction (percentage not specified).

4. Positive Controls: EMS at 0.25 and 0.40 µL/mL and 3-MCA at doses of 2.5 and 4.0 µg/mL were used as the nonactivated and S9-activated positive controls, respectively.

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[1] Only items appropriate to this DER have been included.

5. Media/Growth Conditions: Cells were grown in Fischer's mouse leukemia medium supplemented with 10% horse serum, L-glutamine, sodium pyruvate, antibiotics, and pluronic solution (for maintaining cell integrity). Cloning medium was growth medium plus agar; selection medium was cloning medium containing 3 µg/mL of TFT. All cultures were maintained in a humidified incubator at 37°C in 5% carbon dioxide.

6. Dose Selection: A preliminary cytotoxicity assay (data not submitted) was performed; the authors stated that the test material was only slightly toxic at 5000 µg/mL.

   Based on the results of the preliminary cytotoxicity assay, doses ranging from 50 to 5000 µg/mL were used in the mutation assay. Because 5000 µg/mL is the maximum limit for this assay, higher doses were not tested.

7. Mutagenicity Assay: The assay was performed according to the procedure of Clive and Spector[2] and Clive et al.[3] Logarithmically growing cells seeded at 6×10^6 cells/tube were exposed to the appropriate test material dose, solvent, or positive control with and without S9 activation for 4 hours. Cells were washed, resuspended in growth medium, and reincubated for a 2-day expression period. Daily cell counts were determined; cells were diluted, when appropriate, to a density of 3×10^5 cells/mL to maintain an optimal growth rate. At the end of the expression period, at least five doses were selected for analyses.

   For mutant selection, 1×10^6 cells were seeded into selection medium plates in triplicate. The cloning efficiency (CE) was determined by plating 200 cells/plate (in triplicate) in cloning medium. After 10 to 14 days of incubation, TFT-resistant colonies and total number of viable cells were counted. The MF was calculated by dividing the total number of mutants in each plate by the total number of viable cells and multiplying by 2×10^{-4}.

---

[2]Clive, D. and Spector, J.F.S. Laboratory procedure for assessing specific locus mutations at the TK locus in cultured L5178Y mouse lymphoma cells. Mutat. Res. 31(1975):17-29.

[3]Clive, D., Johnson, K.O., Spector, J.F.S., Batson A.G. and Brown, M.M.M. Validation and characterization of the L5178Y $TK^{+/-}$ mouse lymphoma mutagen assay system. Mutat. Res. 59(1979):61-108.

8. Evaluation Criteria:

a. Assay Acceptability: For the assay to be considered acceptable, the following criteria must be satisfied: 1) the CE of the solvent control should be between 60 and 130%; 2) the average suspension growth of the solvent control value must be at least 8×10^5 for 2 days; 3) the background MF of the solvent control should range from 10×10^{-6} to 110×10^{-6}; 4) positive control values must be within the laboratory's historical controls; 5) the test material must be assayed to a dose that reduces the relative growth to 10-20%; 6) the test material MF can only be evaluated if the relative CE is 10% or greater and the total number of viable clones should exceed 60.

b. Positive Response: The test material was considered positive if it induced a dosed-related increase in MF that was at least 150% of the concurrent background frequency (solvent control) plus 10×10^{-6}.

B. Protocol: See Appendix B.

12. REPORTED RESULTS:

A. Solubility Test: The test material was completely soluble in water (solvent control) at 50 mg/mL. The test material appeared soluble when diluted in the assay medium; no color change was noted.

B. Mutation Assay:

1. Nonactivated System: The test material was assayed, in duplicate cultures, at doses of 50, 300, 1000, 2000, 4000, and 5000 µg/mL without S9 activation. The relative growth ranged from 106.7% at 50 µg/mL to 28.3% at 5000 µg/mL (nondetectable to moderate toxicities). In this assay the minimum criterion for mutagenicity (MCM) was 88.9×10^{-6} (average background frequency of the solvent control, $52.6 \times 150\% + 10 \times 10^{-6}$). At doses from 50 to 4000 µg/mL, the average MFs of 32, 37, 35, 34, and 65×10^{-6}, respectively, did not exceed the MCM; however, at 5000 µg/mL, one duplicate induced a MF of 102×10^{-6}, which exceeded the MCM. The other duplicate culture had an MF of 86.5×10^{-6}, which was not mutagenic. A repeat nonactivated assay was performed to determine if the small increase (1.9-fold increase over the control) was reproducible.

The test material was assayed at 500, 2000, 3000, 4000, 4500, and 5000 µg/mL. The relative growth ranged from 92.4% at 500 µg/mL to 63.3% at 5000 µg/mL (low toxicity). The MCM for this assay was 43.3×10^{-6}. The MF for the test material

doses were 36, 27, 24, 27, 24, and 33 x 10^{-6}, respectively; no increases in MFs were induced. The test material was, therefore, considered nonmutagenic in the absence of S9 activation.

The average nonactivated CE varied from 74.6 to 104.3% (assays 1 and 2, respectively).

2. S9 Activation System: In the presence of S9 activation, doses of 300, 1000, 2000, 3000, 4000, and 5000 µg/mL had relative growths that ranged from 88.3% at 300 µg/mL to 73.6% at 5000 µg/mL (very low toxicity). At the doses tested, the MFs (49, 48, 34, 47, 42, and 46 x 10^{-6}, respectively) of the test material did not exceed the MCM of 72.6 x 10^{-6}. Therefore, the test material in the presence of S9 activation was considered nonmutagenic.

   The average S9 activation CE was 87.3%.

   The solvent control MFs were within the expected range of 10 x 10^{-6} to 110 x 10^{-6}. The positive controls with and without S9 activation induced MFs at levels indicating mutagenic responses.

Representative results are presented in Table 1.

13. STUDY AUTHORS' CONCLUSIONS/QUALITY ASSURANCE MEASURES:

A. The study authors concluded that "The test material, Hoe 039866-Substance Technical, did not induce significant increases in the mutant frequency at the TK locus in L5178Y $TK^{+/-}$ cells. Treatments up to 5000 µg/mL were assayed with and without metabolic activation and, at most, moderate toxicities were induced. Higher concentrations were not assayed because 5000 µg/mL is the testing limit in this assay. None of the assayed treatments induced significant, repeatable increases above the background mutant frequencies (average of solvent controls). The test material is, therefore, considered inactive up to 5000 µg/mL with and without metabolic activation in the Mouse Lymphoma Forward Mutation Assay."

B. A quality assurance statement was signed and dated March 5, 1984.

TABLE 1. Representative Results from the Mouse Lymphoma Forward Mutation Assay with HOE 039866

Substance	Dose/mL	S9 Activation	% Suspension Growth[a]	Total Mutant Colonies	Total Viable Colonies	% Cloning Efficiency[b]	% Relative Growth[c]	Mutation Frequency[d] x 10^{-6}	Fold Increase Over Solvent Control
Solvent Control									
Water[e]	—	-	100	69.5	626	100(104.3)[f]	100	22.2	—
	—	+	100	108.5	534	100 (87.3)[f]	100	41.7	—
Positive Controls									
Ethylmethanesulfonate	0.25 µL	-	84	791	399	66.5	53.5	396.2*	17.8
	0.40 µL	-	63	1111	368	61.2	37.3	609.2*	27.4
3-Methylcholanthrene	2.5 µg	+	57	572	437	72.9	48.0	261.9*	6.3
	4.0 µg	+	52	673	365	60.9	36.8	369.9*	8.9
Test Material									
HOE 039866[g]	500 µg	-	108.2	77.5	519	82.9	89.7	30.1	1.4
	5000 µg	-	64.2	113.5	686	109.7	70.2	33.1	1.5
	300 µg	+	77.6	569	108.6	84.3	84.3	48.6	1.2
	5000 µg	+	66.8	605	115.4	77.0	77.0	46.5	1.1

% Suspension Growth = $\frac{\text{Suspension Growth (test group)}}{\text{Suspension Growth (solvent control)}} \times 100$.

% Cloning Efficiency = $\frac{\text{Cloning Efficiency (test group)}}{\text{Cloning Efficiency (solvent control)}} \times 100$.

% Relative Growth = $\frac{\text{\% Suspension Growth} \times \text{\% Cloning Efficiency}}{100}$.

Mutation Frequency = $\frac{\text{Total Mutant Colonies}}{\text{Total Viable Colonies}} \times 2 \times 10^{-4}$.

Results presented for solvent control were derived from the average of four control cultures; positive controls and test material doses were based on the results of two or three treated cultures.

Number in parentheses is the average absolute cloning efficiency.

Lowest and highest doses tested in the second nonactivate assay and the S9-activated assay. The results of the intermediate doses (2000, 3000, 4000 and 4500 µg/mL-S9; 1000, 2000, 3000, and 4000 µg/mL + S9) were comparable to the solvent control.

Greater than 150% of the mutation frequency of the solvent control plus 10 x 10^{-6} (-S9 > 72.6 x 10^{-6}; +S9 > 72.6 x 10^{-6}), as stated by the study authors.

Reserved for Table 1

14. REVIEWERS' DISCUSSION AND INTERPRETATION OF STUDY RESULTS:

We assess that the study authors interpreted the data correctly and that HOE 039866 assayed at nonactivated doses ranging from 50 to 5000 µg/mL and S9-activated doses ranging from 300 to 5000 µg/mL did not induce a mutagenic response in mouse lymphoma cells. The assay conformed to the laboratory's assay-acceptability criteria and to the recommended testing guidelines.[4] The test material was assayed up to a maximum dose (5000 µg/mL) with or without S9 activation. The positive controls, EMS and 3-MCA, demonstrated the sensitivity of the assay to detect a mutagenic response.

Item 15--see footnote 1.

16. CBI APPENDIX: Appendix A, Materials and Methods, CBI p. 5; Appendix B, Protocol, CBI pp. 12-20.

[4] Federal Register, Vol. 50, No. 188, Subpart F--Genetic Toxicity, Section 798.5300, pp. 39442-39443 (1985).

APPENDIX A

Materials and Methods

RIN 5218-93 / TOX Review for Glufosinate Log #293

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006936

006936

EPA: 68-02-4225
DYNAMAC No. 343-E
February 22, 1988

DATA EVALUATION RECORD

HOE 039866 (IGNITE)

Mutagenicity--In Vitro Chromosome Aberration Study

STUDY IDENTIFICATION: Hirsch, I. E. and Milone, M. F. HOE 039866 substance technical chromosome aberration in cultured human lymphocytes. (Unpublished study No. A30977 prepared by Instituto Di Ricerche Biomediche, "Antoine Marxer" S.p.A., Ivrea, Italy, for Hoechst Celanese Corporation, Sommerville, N.J.; dated February 26, 1985.) Accession No. 403456-13.

APPROVED BY:

Robert J. Weir, Ph.D.
Acting Department Manager
Dynamac Corporation

Signature: Robert J. Weir for

Date: 2-22-88

1. CHEMICAL: Ignite; HOE 039866.

2. TEST MATERIAL: HOE 039866 substance technical, code No. Hoe 039866 OH ZC950 01, was described as a white crystalline powder with a purity of 95.3%. The sponsors stated that the test material was stable under its conditions of use and during the experiment.

3. STUDY/ACTION TYPE: Mutagenicity--In vitro chromosome aberration study in cultured human lymphocytes.

4. STUDY IDENTIFICATION: Hirsch, I. E. and Milone, M. F. HOE 039866 substance technical chromosome aberration in cultured human lymphocytes. (Unpublished study No. A30977 prepared by Instituto Di Ricerche Biomediche, "Antoine Marxer" S.p.A., Ivrea, Italy, for Hoechst Celanese Corporation, Sommerville, N.J.; dated February 26, 1985.) Accession No. 403456-13.

5. REVIEWED BY:

I. Cecil Felkner, Ph.D.
Principal Reviewer
Dynamac Corporation

Signature: I. Cecil Felkner
Date: 2-19-88

Brenda E. Worthy, M.T.
Independent Reviewer
Dynamac Corporation

Signature: Brenda Worthy
Date: 2-19-88

6. APPROVED BY:

Nancy E. McCarroll, B.S.
Genetic Toxicology
Technical Quality Control
Dynamac Corporation

Signature: Nancy E. McCarroll
Date: 2-19-88

Whang Phang, Ph.D.
EPA Reviewer

Signature: Whang Phang
Date: 3/15/88

Marcia Van Gemert, Ph.D.
EPA Section Head

Signature: M. Van Gemert
Date: 3/16/88

7. CONCLUSIONS:

A. Under the conditions of the assay, HOE 039866 substance technical, at doses ranging from 1.0 to 1000 µg/mL, did not induce a clastogenic effect on human lymphocytes with or without S9 activation. The authors demonstrated that the test system was capable of detecting chromosome aberrations because the positive controls, mitomycin C and cyclophosphamide, induced highly significant ($p<0.001$) increases in chromosome aberrations of the lymphocytes in the absence or presence of metabolic activation, respectively.

However, the technical deficiencies listed below preclude acceptance of the results as valid evidence of a negative response:

1. Single cultures of lymphocytes derived from a single donor were used per dose and condition. The recommended approach is either to conduct separate experiments with lymphocytes from different donors or use replicate cultures from different donors in the study.

2. Chromosomes were scored from projected film slides, a procedure that limits the analysis of aberrations to a single plane within the field. The preferred method is to read the slides directly.

3. The slides were not coded.

4. It is not clear how photomicrographs can be taken from labeled slides (not coded) without selecting the slides that are most suitable for presentation (a perceived bias). Further information on this procedure should be provided by the study authors.

Based on the above considerations, we assess that the study authors' conclusions are unsupported.

B. The study is unacceptable.

8. RECOMMENDATIONS:

It is recommended that the study be repeated in accordance with established procedures.[1]

---

[1]Evans, J., and O'Riordan, M. L. Human peripheral blood lymphocytes for the analysis of chromosome aberrations in mutagen tests, Handbook of Mutagenicity Test Procedures, B. J. Kilbey, M. Legator, W. Nichols, C. Ramel, eds., Amsterdam: Elsevier Scientific Publishing Co. (1977), pp. 261-274.

Items 9 through 10--see footnote 2.

11. MATERIALS AND METHODS (PROTOCOLS):

A. Materials and Methods: (See Appendix A for details.)

1. Test Materials: HOE 039866 substance technical, code No. Hoe 039866 OH ZC950001, was described as a white crystalline powder with a purity of 95.3%. The sponsors stated that the test material was stable under the conditions of use. Deionized water was used as the test material solvent and as the negative control.

2. Cell Line: Fresh heparinized blood was obtained from a healthy male volunteer who had not taken any drugs for 30 days prior to the start of study.

3. S9 Fraction: The S9 fraction was prepared from the livers of adult male Sprague-Dawley rats injected intraperitoneally with Aroclor 1254. The S9 homogenate was assayed for protein concentration and for its activation capacity in Salmonella typhimurium strains TA1538, TA98, and TA100 with the promutagen 2-aminofluorene. The S9 mix was prepared according to the method of Ames et al.[3]

4. Positive Controls: The positive controls used in the assay were mitomycin C in the nonactivated system and cyclophosphamide in the S9-activated system.

5. Cytogenetic Assay:

Lymphocyte Preparation: Stimulation of lymphocytes was achieved by mixing heparinized human blood with culture medium containing the mitogen phytohemagglutinin. The cultures were incubated for 48 hours at 37° C.

Exposure: Single prepared cultures were incubated with 1, 10, 100, or 1000 µg/mL of the test material and with the positive or solvent control for 3 hours at 37° C with or without S9 activation. The cultures were centrifuged, and the pellets were resuspended in fresh culture medium and incubated for 23 hours; colchicine was added and the cultures were incubated for an additional 3 hours. Cells were

[2]Only items appropriate to this DER have been included.

[3]B. N. Ames et al. Methods for detecting carcinogens and mutagens with Salmonella/mammalian-microsome mutagenicity test, Mutat. Res. 31 (1975): 347-364.

recovered as pellets and suspended in 0.5 mL of a cold-fixing mixture, placed onto slides, dried, and stained.

Slide Analysis: Two slides of each of the test material and the positive and solvent controls were prepared and labeled with the experiment number, a number indicating the treatment/dose level, and an identification letter. Slides were photomicrographed (1250x magnification), and at least 100 metaphases per culture were analyzed. Only those metaphases having well-distributed and morphologically identifiable chromosomes were examined. Chromosome aberrations were classified according to the criteria of Evans and O'Riordan[4] and Killian et al.[5]

Statistical significance at three levels ($p < 0.05$, $p < 0.01$, and $p < 0.001$) was assessed using the chi-square method to compare the incidence of chromosome aberrations in the test material doses or the positive control to the solvent control.

12. REPORTED RESULTS:

A. A preliminary cytotoxicity assay was conducted with HOE 039866 substance technical at doses of 1, 10, 100 and 1000 µg/mL. Results showed a dose related reduction in mitotic activity; activity ranged from 93 percent to 55 percent of the control values at 1 and 1000 µg/mL, respectively. The report did not specify whether S9 activation was used in this assay.

B. Cytogenetic Assay:

1. Nonactivated Assay: At four doses ranging from 1 to 1,000 µg/mL, there were no increases in aberrations, with or without gap when compared to the negative control (deionized water). These results were from examining more than 90 metaphases at each dose (at least 100 metaphases were prepared and photographed for examination at each dose of the test material and the positive and negative controls); the actual number of scorable slides were dependent upon test limits, toxicity, and the related technical limitations. Representative results from these data are presented in Table 1.

---

[4] Ibid.

[5] Killian, P. J., Moreland, F. M., Benge, M. C., Legator, M. S., Whorton, Jr., E. B. A collaborative study to measure interlaboratory variation with the in vivo bone marrow metaphase procedure, Handbook of Mutagenicity Procedures, B. J. Kilbey, M. Legar, W. Nichols, C. Ramel, eds., Amsterdam: Elsevier Scientific Publishing Co. (1977)' pp. 243-260.

TABLE 1. Representative Results of Chromosome Aberrations in Human Lymphocytes with HOE 039866 - Substance Technical

Substance	Dose (µg/mL)	S9 Activation	No. of Metaphases Counted	Total Aberrations (%) Including Gaps	Total Aberration (%) Excluding Gaps	Mitotic[a] Activity (%)
Solvent Control						
Deionized Water	0	-	97	2(2.06)	1(1.03)	100
	0	+	102	1(0.98)	0(0)	-
Positive Controls						
Mitomycin C	2	-	106	40(37.74)*	36(34.00)*	-
Cyclophosphamide	35	+	99	29(29.30)*	19(19.20)*	-
Test Material[b]						
HOE 039866	100	-	99	1(1.01)	1(1.01)	69
	1000	-	91	0	0	55
	100	+	99	1(1.01)	0	-
	1000	+	91	1(1.01)	0	-

[a]Decreased mitotic activity indicates cytotoxicity, probably in the absence of S9 but not specified by the authors.

[b]Results are for the two highest doses tested; findings for the lower doses (1 and 10 µg/mL) were comparable to the solvent control.

*Statistically significant from control values at $p < 0.001$.

2. S9-Activated Assay: The same doses used in the nonactivated assay were also used in the S9-activated assay, and there were no increases in aberrations, with or without gaps. Representative results from these data are presented in Table 1.

3. Positive Controls With and Without S9 Activation: The non-activated control, 2 µg/mL mitomycin C, caused a significant increase ($p<0.001$) in the percent of total aberrations, with or without gaps, when compared to the negative control. The S9-activated control, 35 µg/mL cyclophosphamide, also caused a significant increase ($p<0.001$) in the percent of total aberrations, with or without gaps, when compared to the negative control. These data are also presented in Table 1.

13. STUDY AUTHORS' CONCLUSIONS/QUALITY ASSURANCE MEASURES:

A. "The results of this study show that the test article HOE 039866-SUBSTANCE TECHNICAL up to the concentration of 1000 µg/mL did not induce statistically significant increase in chromosome aberration in cultured human lymphocytes either in the presence or absence of metabolic activation."

B. A quality assurance statement was signed and dated March 11, 1985.

14. REVIEWERS' DISCUSSION AND INTERPRETATION OF STUDY RESULTS:

We assess that the dosing with HOE 039866 was at sufficiently high levels to ensure cytotoxicity; this was indicated by data showing suppression of mitotic activity at 100 and 1000 µg/mL.

However, there are some serious reporting and technical deficiencies, which compromise the study. The toxicity test clearly showed that the test material affected mitotic activity, but the authors failed to state whether the reported data was from the nonactivated or S9-activated assay; both nonactivated and S9 activated cytotoxicity should have been included. The assay was performed with single cultures for each experimental point and the lymphocytes were derived from one individual. At least two replicate cultures should be used for each dose group and it is advisable to use blood samples from at least two donors to minimize the possibility of varying responses between the lymphocytes of different individuals. The study authors scored aberrations from projected film slides, a procedure that limits the analysis to a single plane; the preferred method is to score slides directly. In addition, slides were not coded.

APPENDIX A

Materials and Methods

RIN 5218-93 / Tox Review for Glufosinate Log #293

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Reviewed by: Whang Phang, Ph.D. 8/16/88
Secondary Reviewer: Marcia van Gemert, Ph.D. 8/17/88
Section III, Tox. Branch (TS-769c)

DATA EVALUATION REPORT

006936

STUDY TYPE: In vitro assays of the effects of Hoe 039866 on various enzymes

CHEMICAL: Hoe 039866 (Ignite); Hoe 035956

EPA ACCESSION No: 403456-36 CASWELL No.: 580I

EPA ID No: 8340-EO/8340-EI EPA PROJECT No: 8-0146

SPONSOR: Hoechst Celanese Corp.

TESTING LABORATORY: Hoechst AG, 6230 Frankfurt am Main 80, Federal Republic of Germany

CITATION: Kocher, H. (1986). Investigating the effect of the herbicides Hoe 039866 and Hoe 035956 on various enzymes in vitro. Hoechst AG, Germany; Report No.: A34404; Project No.: Ko 3/1986. June 23, 1986. Submitted by Hoechst Celanese Corp., Sommerville, NJ. Aug 27, 1987.

METHODS AND MATERIALS:

Test articles: Hoe 039866 and Hoe 035956 (purity not specified).

Enzymes: glutamate oxalacetate transaminase (GOT), glutamate pyruvate transaminase (GPT), glutamate dehydrogenase (GDH), and gamma-glutamyl transpeptidase (GGTP) were purchased commercially as kits from Sigma.

Procedures: The details of the procedures used in these assays are excerpted from the report and presented in the Appendix. They will not be repeated here.

RESULTS:

Both 10 mmol/L Hoe 039866 and Hoe 035956 did not influence the enzymic activity of GOT, GPT, and GGTP at all (Tables 1, 2, & 3).

In Table 4, the mean value of the assays containing Hoe 039866 and that of Hoe 035956 was interchanged.

The mean value of GDH activity appeared to indicate that the test agents slightly decreased this enzyme activity. However, the individual assay results indicate that one value of the control assay was substantially higher than the others (Table 4), and it seemed to be an outlier. The other values were comparable to those of Hoe 039866 containing assays. The inhibitory effect Hoe 035956 was shown to be greater than that of Hoe 035956 (Table 4).

DISCUSSION AND SUMMARY:

Based upon the results presented in this report, 10 mmol/L Hoe 039866 did not inhibit the enzymic activity of GOT, GPT, and GGTP. Although 10 mmol/L Hoe 039866 appeared to slightly inhibit GDH activity, but the inhibition was so small. In addition, one of the control values seemed to be an outlier. Based upon the reported data, the inhibitory effect of 10 mmol/L Hoe 039866 on GDH was shown to be very small.

This study provides some supplementary information, but it does not meet the toxicology study requirements. It is classified as Supplementary.

RIN 5218-93 / TOX Review for Glufosinate Log #293

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Reviewed by: Whang Phang, Ph.D. 8/10/88
Secondary Reviewer: Marcia van Gemert, Ph.D. 5/15/88
Section III, Tox. Branch (TS-769c)

DATA EVALUATION REPORT

006936

STUDY TYPE: Catecholamines and glutamine synthetase determinations on Hoe 039866 & Hoe 061517 treated rats

CHEMICAL: HOE 039866; monoammonium [2-amino-4-(hydroxymethyl phosphinyl)butanoate]; Ignite®

006936

EPA ACCESSION No: 403456-27

CASWELL No.: 580I

EPA ID No: 8340-EO/8340-EI

EPA PROJECT No: 8-0146

SPONSOR: Hoechst Celanese Corp.

TESTING LABORATORY: Hoechst AG, 6230 Frankfurt AM Main 80
Federal Republic of Germany

CITATION: Gerhards, H. (1986). Pharmacological screening of Hoe 039866-substnace technical (Code: Hoe 039866 OH ZC97 0001) and Hoe 061517-substance technical (Code: Hoe 061517 OQ ZD97 0002) following I.C.V. or I.V. applicaton in male Wistar rats. I. Behavioural assessment and determination of catecholamines in various regions of the rat brain. II. Determination of glutamine synthetase activity in the rat brain. Hoechst AG; Report No.: A34318; Project No.: 86.1190; Sept 25, 1986. Submitted by Hoechst Celanese Corp., Sommerville, NJ. Aug 27, 1987.

SUMMARY: In Wistar male rat, intracerebral (i.c.v.) injection of 20 ug Hoe 039866 caused severe convulsions which were reported to be ameliorated by diazepam (10 mg/kg, i.p.). A dose of 10 ug Hoe 039866 (i.c.v.) also induced spasm of forelimbs.

With i.c.v. injection of 20 ug Hoe 061517, a major metabolite of the test agent, spasm of forelimbs was observed.

Changes in brain catecholamines were observed in rats which were treated with 20 ug Hoe 039866 (i.c.v. injection), but the biological significance of these changes were not clear.

With i.c.v. injection, Hoe 039866 at doses of 10 and 20 ug significantly inhibited brain glutamine synthetase activity, and the inhibition was dose-dependent.

Intravenous injection of the test agent at doses of 10 and 100 mg/kg did not produce significant changes in the levels of brain catecholamines or of glutamine sythetase activity.

This study provides clear evidence that the test agent caused convulsion and innibited brain glutamine synthetase activity, when it entered the central nervous system. However, the study does not meet the guidelines for a toxicology study; in addition, the report has no individual animal data on catecholamine determinations. The study is, therefore, classified as Supplementary.

METHODS AND MATERIALS:

Test chemicals: Technical grade Hoe 039866; purity, 96.9%
Technical grade Hoe 061517, a metabolite of Hoe 039866; purity, 99%

Animals: Wistar rats
mice (strain not specified)

Experimental procedure: In a preliminary study, 8 male Wistar rats (260-310 gm body weight) were implanted with ventricular catheter for intracerebral (i.c.v.) injection. The animals received 10 or 20 ug Hoe 039866 or Hoe 061517 i.c.v. injection and followed by a 24 hour (hr) observation period.

In the main study, groups of animals (6/dose/group) were implanted with ventricular catheters and infused with 10 or 20 ug Hoe 039866, 20 ug Hoe 061517, or 10 ul physiological saline. the animals were sacrificed 3 hrs after treatment; brain of each animal was removed for determination of catecholamines and glutamine synthetase activity. In a parallel study, 15 rats were injected (i.v.) with 10 or 100 mg/kg Hoe 039866. After sacrifice, the brain of each animal was removed for determination of catecholamines and glutamine synthetase activity.

The analytical methods for catecholamines and glutamine synthetase were excerpted from the submitted report and presented in the Appendix.

RESULTS:

Preliminary study: Animals which received 20 ug Hoe 039866 by i.c.v. injection went into convulsion 3-4 hrs after treatment (Table 1a). The convulsions were found to be ameliorated with diazepam at a dose of 10 mg/kg (i.p.). A dose of 10 ug Hoe 039866 produced relatively slight spasm of the forelimbs and opisthotonus which disappeared with injection of diazepam (10 mg/kg, i.p.).

The metabolite, Hoe 061517, at a dose of 20 ug caused clonic spasm of forelimbs, but at 10 ug no behavioral or motor anomalies were observed in the 2 treated animals (Table 1b).

Main study:

Brain catecholamine levels:

It should be noted that no individual animal data on catecholamine determinations were presented in the report, and the reported data on catecholamine levels could not be verified.

In rats which received i.c.v. injection, catecholamine determinations were conducted on tissues of frontal cortex, striatum, and hippocampus. In 20 ug Hoe 039866 treated rats, a 31% decrease in noradrenalin (NA) in frontal cortex, a 63% increase in dihydroxyphenyl acetic acid (DOPAC) in striatum, a slight decrease in NA of hippocampus were observed (Table 2). The decrease of NA in frontal cortex and the increase of DOPAC in striatum were statistically significant.

In 10 ug Hoe 039866 rats, no marked changes in the catecholamine levels were seen (Table 2).

In 20 ug Hoe 061517 treated rats, a decrease in dopamine (DA) in striatum was observed, but this decrease was not statistically significant. Other values were comparable to those of the controls (Table 2).

In animals which received intravenous injections of Hoe 039866, no statistically significant changes in catecholamine level were reported (Table 3). However, there was a decrease in NA in striatum of 100 mg/kg rats.

Brain Glutamine synthetase activity: Intravenous injection of Hoe 039866 at doses of 0, 10, and 100 mg/kg did not produce inhibition of glutamine synthetase activity (Table 4). Although the mean of 100 mg/kg group showed a decrease in enzyme activity, this decrease was mainly due to a single animal.

The brain glutamine synthetase activity was inhibited in rats which were treated with i.v.c. injection of 10 and 20 ug Hoe 039866 (Table 5). The decreases in glutamine synthetase activity was statistically significant ($p < 0.001$) and dose-dependent. The metabolite, Hoe 061517, at 20 ug (i.c.v. injection) did not cause any change in brain glutamine synthetase activity (Table 5).

DISCUSSION:

In a preliminary study, severe convulsions were observed in male rats which received i.c.v. injection of 20 ug Hoe 039866. Diazepam (10 mg/kg, i.p.) was reported to ameliorate the convulsions produced by the test agent. Mild consulsion was also observed in rats treated with 10 ug Hoe 039866 (i.c.v. injection).

A major metabolite of Hoe 039866, Hoe 061517, at 20 ug also caused clonic spasm of forelimbs.

There were statistically significant changes in catecholamine levels in certain areas of the brain of 20 ug Hoe 039866 treated (i.c.v.) rats, namely a 31% decrease in NA in frontal cortex and and a 63% increase in DOPAC in striatum. The biological significance of these changes was not clear. The metabolite, Hoe 061517, did not markedly affect the levels of brain catecholamines.

Intravenous injection of the test agent at doses of 10 and 100 mg/kg did not produce significant changes in the levels of brain catecholamines or of glutamine sythetase activity.

With i.c.v. injection, Hoe 039866 at doses of 10 and 20 ug significantly inhibited brain glutamine synthetase activity, and the inhibition was dose-dependent.

This study provides clear evidence that the test agent caused convulsion and inhibites brain glutamine synthetase activity, when it enters the central nervous system. However, the study does not meet the guidelines for a toxicology study; in addition, the report has no individual animal data on catecholamine determinations. The study is, therefore, classified as Supplementary.

RIN 5218-93 / Tox Review for Glufosinate Log # 293

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Reviewed by: Whang Phang, Ph.D. 8/3/88 006936
Secondary Reviewer: Marcia van Gemert, Ph.D. 8/4/88
Section III, Tox. Branch (TS-769c)

DATA EVALUATION REPORT

STUDY TYPE: Testing the therapeutic effects of atropine sulphate and 2-PAM on Hoe 039866 treated rats 006936

CHEMICAL: HOE 039866; monoammonium [2-amino-4-(hydroxymethylphosphinyl)butanoate]; Ignite®

EPA ACCESSION No: 403456-28 CASWELL No.: 580I

EPA ID No: 8340-EO/8340-EI EPA PROJECT No: 8-0146

SPONSOR: Hoechst Celanese Corp.

TESTING LABORATORY: Hoechst AG, 6230 Frankfurt AM Main 80
Federal Republic of Germany

CITATION: Ebert, E. and Leist, K. -H. (1986). Hoe 039866-active ingredient technical (Code: Hoe 039866 OH ZC95 0001): Testing the therapeutic effect of atropine sulphate and 2-PAM methiodide after acute intoxication with Hoe 039866 in the male and female Wistar rat. Hoechst AG; Report No.:A34188; Project No.:86.0973; August 27, 1986. Submitted by Hoechst Celanese Corp., Somerville, NJ. Aug 27, 1987.

SUMMARY: Atropine sulphate and 2-PAM-methiodide did not produce any therapeutic effect on rats which were treated with lethal dose of Hoe 039866 (3200 and 2200 mg/kg for males and females, respectively).

This study provides useful information, but it is a special study. Therefore, it is classified as Supplementary.

Methods and Materials:

Test chemicals: Technical grade Hoe 039866; purity, 95.3%; white powder.

Atropine sulphate was manufactured by Merck AG, Darmstadt (FRG). Solution for injection was 1% (w/v) in sterile water.

2-PAM-methiodide was manufactured by Sigma Chemicals, St. Louis (USA). Solution for injection was 7.5% (w/v) in sterile water.

Animals: Hoe: WISKF(SPF71) rats were obtained from Hoechst AG, Kastengrund, SPF breeding colony. These rats were approximately 8-10 weeks old with mean weights of 199 (male) and 182 (female).

006936

Procedures: An earlier study indicated that intraperitoneal administration of atropine sulphate (10 mg/kg) and 2-PAM-methiodide (75 mg/kg) did not reverse the effects of Hoe 039866 (3200 mg/kg for male and 2200 mg/kg for females). The present study was conducted to verify the previous findings with lower doses of the atropine and 2-PAM as follows:

TABLE 1*

Group	Number of animals	Hoe 039866 (mg/kg b.w.)	Therapeutic agent	Dose (mg/kg b.w.)	Route of admin.
Males					
1	10	3200	---	---	---
2	10	3200	atropine sulphate	5	i.p.
			2-PAM-methiodide	37.5	i.p.
Females					
1	10	2200	---	---	---
2	10	2200	atropine sulphate	5	i.p.
			2-PAM-methiodide	37.5	i.p.

* Data excerpted from the submitted report (Hoechst Report No. A34188)

Rats (20/sex) were administered (by gavage) Hoe 039866 as indicated in Table 1. Groups of 10 rats/sex was then treated with atropine sulphate (5 mg/kg) and 2-PAM-methiodide (37.5 mg/kg) according to the following schedules:

TABLE 2*

Group	Time after treatment 0	4h	6h	8h	23h	31h
Males						
1	T	-	-	-	-	-
2	T	+o	o	+o	+o	-
Females	T	-	-	-	-	-
1	T	-	-	-	-	-
2	T	+o	o	+o	+o	+o

T = Hoe 039866 active ingredient technical
\+ = 2-PAM-methiodide (37.5 mg/kg body weight)
o = atropine sulphate (5 mg/kg body weight)

* Data excerpted from the submitted report (Hoechst Report No.: A34188).

The animals were weighed weekly and observed for 14 days. Rats, which were sacrificed, were dissected and examined macroscopically.

RESULTS:

1). Mortality and approximate time of death are presented in Table 3.

TABLE 3†

Group	Days after treatment* 0d - 1d - 2d - 3d - 4d - 5d - 6d - 7d - 8d	Total 14d
Males		
1 (control)	6 1 1 1	10/10
2 (2-PAM + AS)	5 1 1 1 1 1**	10/10
Females		
1 (control)	1 4 2 1	8/10
2 (2-PAM + AS)	1 4 2 1 1 1	10/10

* day 0 = day of treatment with Hoe 039866 active ingredient technical
** animal removed from study in moribund condition

† Data excerpted from the submitted report (Hoechst Report . No.: A34188)

The results indicated that administration of atropine and 2-PAM-methiodide did not protect the animals from the toxic effects of Hoe 039866. The toxic effects include hypersensitivity, drowsiness, tono-clonic convulsions, generalized tremors, rolling spasms, salivation, isolated dacryorrhea, and diarrhea.

The animals, which were found dead, showed reddish brown to dark brown adrenals and hemorrhage in the region of small intestine. The two surviving no-therapy females did not show any abnormal macroscopic findings.

DISCUSSION:

Following Hoe 039866 administration, atropine sulphate and 2-PAM-methiodide treatment did not protect the animals from the effects of the test agent.

Reviewed by: Whang Phang, Ph.D. 8/15/88
Secondary Reviewer: Marcia van Gemert, Ph.D. 8/15/88
Section III, Tox. Branch (TS-769c)

DATA EVALUATION REPORT (DER)

006936

STUDY TYPE: General pharmacology study with Hoe 039866

006936

CHEMICAL: Ignite®; Hoe 039866 (97.0% purity, with lot No. of 12640)

EPA ACCESSION No: 403456-24 CASWELL No.: 580I

EPA ID No: 8340-EO/8340-EI EPA PROJECT No: 8-0146

SPONSOR: HJL on behalf of Hoechst AG

TESTING LABORATORY: Institute of Environmental Toxicology
Tokyo, Japan

CITATION: Takahashi, H. et al. (1986). Hoe 039866 OH ZC 97 0003: General pharmacology study. The Institute of Environmental Toxicology; Hoechst AG; Report No.: A34071; March 24, 1986. Submitted by Hoechst Celanese Corp., Sommerville, NJ. Aug 27, 1987.

DISCUSSION AND SUMMARY:

This report contains several studies some of which can be considered as parts of a chronic toxicity study or a neurotoxicity study while others are irrelevant to toxicology. This reviewer has evaluated and verified the results, and found the summary to be accurate. A detailed DER will not prepared for this submitted report, but the summary section and the table of contents are excerpted from the report and presented along with this abbreviated DER.

RIN 5218-93 / TOX Review for Glufosinate Log #293

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Pages 559 through 564 are not included.

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Reviewed by: Whang Phang, Ph.D. 8/2/88
Secondary Reviewer: Marcia van Gemert, Ph.D. 8/4/88
Section III, Fox. Branch (TS-769c)

DATA EVALUATION REPORT (DER)

006936

STUDY TYPE: In-vitro receptor binding assays

CHEMICAL: Monoammonium [2-amino-4-(hydroxymethylphosphinyl)-butanoate]; Hoe 039866 liquid concentrate 40; Ignite®

EPA ACCESSION No: 403456-30 CASWELL No.: 580I

EPA ID No: 8340-EO/8340-EI EPA PROJECT No: 8-0146

SPONSOR: Hoechst Celanese Corp.

TESTING LABORATORY: Hoechst AG, 6230 Frankfurt AM Main 80
Federal Republic of Germany

CITATION: Schacht, U. (1986). Hoe 039866 substance technical (Code: Hoe 039866 OH ZD97 0001) testing the mode of action by neurotransmitter receptor binding assays in-vitro. Hoechst AG; Report No.: A34303; Project No.: 86.1243; Oct 3, 1986. Submitted by Hoechst Celanese Corp., Sommerville, NJ. Aug 27, 1987.

SUMMARY:

The results of the receptor binding assays indicated that 1 uM Hoe 039866 did not bind significantly with any of the receptors examined (GABA, NA-alph$_2$, NA-beta, 5-HT$_1$, 5-HT$_2$, Ca^{++} channel, and benzodiazepine receptors).

The assays were conducted well. However, this study is a special study which provides supplementary information about Hoe 039866, and the study is considered as Supplementary.

METHODS AND MATERIALS:

Test chemical: Hoe 039866 (Code: Hoe 039866 OH ZD97 0001); 96.9% purity

Membrane fractions: The receptor containing membrane fractions were isolated from rat brain for gamma-aminobutyrate (GABA), noradrenal (NA), seritonin (5-HT), and Ca^+ channel were derived from rat brain. The membrane fractions of benzodiazepine and dopamine were obtain from bovine brain.

Procedures: The experimental procedures and the reaction conditions were excerpted from the submitted report and presented in the Appendix. They will not be repeated here.

RESULTS:

The data on radioactivity measurements of the ligand and on the ligand with addition of 1 uM Hoe 039866 indicated that Hoe 039866 had very little binding affinity for all the receptors examined (Table 2). Although 1 uM Hoe 039866 produced a 7% displacement of ^{3}H-muscimol for the GABA receptor binding, this slight displacement could not be considered as biologically significant.

DISCISSION

The results of the receptor binding assays indicated that 1 uM Hoe 039866 did not bind significantly with any of the receptors examined.

RIN 5218-93 / TOX Review for Glufosinate Log #293

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Pages 567 through 569 are not included.

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Reviewed by: Whang Phang, Ph.D. 9/19/88
Section III, Tox. Branch (TS-769C)
Secondary reviewer: Marcia van Gemert, Ph.D. 9/19/88
Section III, Tox. Branch (TS-769C)

DATA EVALUATION REPORT

006936

STUDY TYPE: Determination of glutamine synthetase (GS) activity, NH_4^+, glutamine, and glutamate levels in brain, liver, kidney, and heart of rats and mice

CHEMICAL: HOE 039866; Monoammonium [2-amino-4-(hydroxymethyl-phosphinyl)butanoate]; Ignite® 006936

ACCESSION NUMBER: 403456-23 CASWELL NO.: 580I

EPA ID NO: 8340-EO/8340-EI EPA PROJECT NO: 8-0146

SPONSOR: Hoechst Celanese Corp.

TESTING FACILITY: Hoechst AG, 6230 Frankfurt am Main 80 Federal Republic of Germany

CITATION: Ebert, E. and Kramer, M.. HOE 039866-- Active ingredient Technical (Code: HOE 039866 OH ZC95 0001)- Examination of the mode of action following a single administration at doses up to the sublethal range to female Wistar rats and female NMRI mice; determination of glutamine synthetase activity and of the substrate levels of NH_4^+, glutamine, and glutamate in various organs. Hoechst AG: Report No. A34503 Project No. 85.0066. May 7, 1985. Submitted by Hoechst Celanese Corp., Somerville, NJ.

CONCLUSION: Groups of 5 female rats or mice were gavaged with with technical HOE 039866 at doses of 50 and 200 mg/kg for mice and 200 and 800 mg/kg for rats; the animals were sacrificed 4 hrs after dosing. The following findings were obtained:

1). No clinical signs of toxicity were observed in treated animals.

2). In general, in all organs examined the level of GS activity was higher in mice than that in rats. No changes in brain GS activity was found in both rats and mice. Significant decrease in kidney GS activity was seen in all treated rats and mice. Reduced liver GS activity was also found in 800 mg/kg rats. In contrast, heart GS activity was slightly elevated in mouse.

3). The NH_4^+ level in most organs examined in female rats and mice was comparable between treated and control animals except in the liver of 200 mg/kg female mice which show a slight increase.

4). No changes were observed in glutamine and glutamate levels in all organs examined in rats and mice.

This study has been properly conducted, and provides useful information. However, the study used only female animals, and no explanation was offered for this selective experimental condition. The study is classified as supplementary.

006936

METHOD AND MATERIALS:

1). Test compound: Hoe 039866, technical 95.3% pure (Ignite®); white crystalline powder; Code: Hoe 039866 OH ZC95 0001

2). Animals (species/strain): Females

Wistar rats/Hoe: WISKf (SPF71)
mean of body weight: 203 ± 8.9 gm

NMRI mice/Hoe: NMRKf(SPF71)
mean of body weight: 17.7 ± 1.0 gm

These animals were obtained from Hoechst AG, Kastengrund

3). Experimental procedures:

The acute oral toxicity studies on female rats and mice (Report Nos.: 80.0588 and 80.0546) yielded the LD_{50} values of 1600 and 400 mg/kg for female rats and mice, respectively. Based upon these values the following doses were selected:

Dose (mg/kg)	Concent. % (w/v)	Vol. applied (ml/kg)	No. of animals rats	mice
0 (water)	0	10	5	5
50	0.5	10	-	5
200	2.0	10	5	5
800	8.0	10	5	-

Data excerpted from the submitted report.

Following the administration (by gavage) of the test agent the animals were observed for any signs of intoxication and death. They were sacrificed 4 hrs after treatment, and the following organs were removed, weighed, and fixed in liquid nitrogen for biochemical determination of the following parameters:

- glutamine synthetase activity
- NH_4^+ level
- glutamine level
- glutamate level

Due to limited amounts of mouse tissues available, the levels glutamine and glutamate were not determined for mice.

The details of biochemical analyses are presented in the Appendix 1. The numerical results were evaluated with Dunnett test. The report also stated that organ weights were analyzed by Standard Evaluation of One-Way Classified Data-Version 0.

RESULTS:

a). Clinical observations: No signs of acute toxicity were observed in all treated females mice or rats. This finding was consistent with that seen in the acute oral toxicity studies at these dose levels (50 and 200 mg/kg for mice; 200 and 800 mg/kg).

b). Glutamine synthetase activity: The glutamine synthetase (GS) activity varied considerably from one organ to the other as indicated by the control means of both mice and rats (Table 1). The brain appeared to contain the highest level among all the organs examined in both rodent species. In general, GS activity was substantially higher in all the organs examined in mice than that in rats (Table 1).

For mice, brain GS activity was comparable between treated and control animals (Table 1a). Statistically significant decrease in kidney GS activity was observed in treated animals. In contrast, an increase in GS activity was found in the heart of treated mice relative to that of the controls (Table 1a).

For rats, little difference was found in brain GS activity between treated and control animals (Table 1b). As in mice, there were decreases in kidney and liver GS activities; these decreases were statistically significant except for the decrease in liver GS activity in 200 mg/kg (Table 1b). The GS activity of the heart between treated and control animals was comparable.

c). NH_4^+ Levels: The NH_4^+ levels in brain, liver, kidneys, and hearts were comparable between treated and control rat (Table 2a).

In mice, there was a slight and statistically significant increase in liver NH^+_4 level in 200 mg/kg group; the NH^+_4 levels of other organs showed no difference between treated and control animals (Table 2b).

d). Glutamine and glutamic acid levels in rat tissues: There was no change in glutamine and glutamic acid levels in brain and liver of treated and control rats (Table 3).

DISCUSSION:

Groups of 5 female rats or mice were gavaged with technical HOE 039866 at doses of 50 and 200 mg/kg for mice and 200 and 800 mg/kg for rats; the animals were sacrificed 4 hrs after dosing. No clinical signs of toxicity were observed in treated animal.

In general, in all organs examined the level of GS activity was higher in mice that that in mice. No changes in brain GS activity were found in both rats and mice. Significant decrease in kidney GS activity was seen in all treated rats and mice. Reduced liver GS activity was also found in 800 mg/kg rats. In contrast, heart GS activity was slightly elevated in mouse.

The NH_4^+ level in most organs examined in rats and mice was comparable between treated and control animals except in the liver of 200 mg/kg mice which show a slight increase.

No changes were observed in glutamine and glutamate levels in all organs examined in rats.

Under the experimental conditions, oral administration of HOE 039866 at sublethal doses did not cause any changes in the levels of GS activity, NH_4^+, glutamine, and glutamate in the brain of rats and mice.

RIN 5218-93 / Tox Review for Glufosinate Log #293

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Pages 574 through 580 are not included.

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Reviewed by: Whang Phang, Ph.D. 8/31/88
Secondary Reviewer: Marcia van Gemert, Ph.D.
Section III, Tox. Branch (TS-769c) 8/31/88

006936

DATA EVALUATION REPORT

STUDY TYPE: Effect of Hoe 039866 on the function of rat liver mitochondria (in vitro)

CHEMICAL: Ignite®; Hoe 039866 (99.5% purity, with lot No. of OH ZB99 0002)

EPA ACCESSION No: 403456-35 CASWELL No.: 580I

EPA ID No: 8340-EO/8340-EI EPA PROJECT No: 8-0146

SPONSOR: Hoechst Celanese Corp.

TESTING LABORATORY: Hoechst AG, Federal Republic of Germany

CITATION: Metzger, H. (1986). Hoe 039866- pure active ingredient (Code: Hoe 039866 OH ZB99 0002) action on rat liver mitochondria. Hoechst AG; Report No.: A34359; Sept 19, 1986. Submitted by Hoechst Celanese Corp., Sommerville, NJ. Aug 27, 1987.

MATERIALS AND METHODS:

Test article: Hoe 039866, a.i.; purity 99.5% with lot No. of OH ZB99 0002

Experimental procedures: The experimental procedures for this study are excerpted from the report and presented in the Appendix; they will not be repeated here.

RESULTS:

Oxidative phosphorylation: The results indicated that the test agent did not influence the rat liver mitochondria function as measured by P/O - coefficient, respiratory control, oxygen consumption, and decoupling of oxidative phosphorylation (Tables 1, 2, 3, & 4).

DISCUSSION AND SUMMARY:

Under the present experimental conditions, Hoe 039866 either did not affect the function of rat liver mitochondria or it could not be taken up by this organelle. Although this study provides useful information, it does not meet any toxicology study guidelines. It is classified as Supplementary.

RIN 5218-93 / Tox Review for Glufosinate Log # 293

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Reviewed by: Whang Phang, Ph.D. 8/25/88
Secondary Reviewer: Marcia van Gemert, Ph.D. 9/6/88
Section III, Tox. Branch (TS-769c)

DATA EVALUATION REPORT

STUDY TYPE: Inhibition of liver glutamine synthetase by phosphonic analogues of glutamic acid 006936

CHEMICAL: phosphonic analogues of glutamic acid (for structures, see the attached article)

EPA ACCESSION No: 403456-34 CASWELL No.: 580I

EPA ID No: 8340-EO/8340-EI EPA PROJECT No: 8-0146

SPONSOR: Published study in Experientia 37: 461-462 (1981) (Attachment)

TESTING LABORATORY: Institute of Organic and Physical Chemistry Technical University, Poland

CITATION: Lejczak, B., Starzemska, H., and Mastalerz, P. (1980). Inhibition of rat liver glutamine synthetase by phosphonic analogues of glutamine acid. Technic University, Poland; Report No.: A32191; Sept 25, 1980. Submitted by Hoechst Celanese Corp., Sommerville, NJ; Aug 27, 1987.

SUMMARY: The relevant results indicate that analogues of glutamic acid and glutamine in which alpha- or gamma-COOH groups were substituted by PO_3H_2 or $P(O)(CH_3)OH$ groups competitively inhibited rat liver glutamine synthetase activity (Attachment). However, the report is a published article, and the results can not be verified. The study is Unacceptable.

Experientia 37 (1981), Birkhäuser Verlag, Basel (Schweiz)

Inhibition of rat liver glutamine synthetase by phosphonic analogues of glutamic acid[1] *

B. Lejczak, H. Starzemska and P. Mastalerz

Institute of Organic and Physical Chemistry, Technical University, PL-50370 Wrocław (Poland), 25 September 1980

Summary. Analogues of glutamic acid, α-methylglutamic acid and glutamine in which the α- or γ-COOH groups are replaced by PO_3H_2 or P(O)(OH)CH$_3$ functions competitively inhibit rat liver glutamine synthetase. The K_i values are comparable to or lower than K_M for L-glutamate.

Aminophosphonates related structurally to glutamic acid appear to be of some interest as biologically active substances. Thus, phosphinothricin (2, table) is a natural compound with antibiotic properties[2,3], while 2-amino-4-phosphonobutyric acid (1) competes with glutamate for receptors in nerve cells[4,5] and has been reported to have antiviral activity[6].

The mode of antibacterial action of phosphinothricin involves inhibition of glutamine synthesis in the bacterial cell by competition with glutamic acid[2]. Considering this fact, and the much earlier observation that 1 and its P-ethyl analogue are strong inhibitors of pigeon liver glutamine synthetase[7], it appeared worthwhile to extend the number of phosphonic analogues of glutamic acid examined for inhibitory properties in glutamine synthesizing enzyme systems. In this communication we report the inhibition of rat liver glutamine synthetase by phosphonic and P-methylphosphinic acids structurally related to glutamic acid, α-methylglutamic acid, glutamine and isoglutamine (table).

All analogues listed in the table were prepared in our laboratory in the form of racemic mixtures[8-11] and were used as such in enzyme tests.

The inhibition was studied using glutamine synthetase (EC.6.3.1.2) prepared from rat liver[12]. Enzyme activity with and without inhibitors was assayed by the hydroxamate procedure[13] in which K_M for L-glutamate was 2.4×10^{-3} M. Assays were conducted at 37 °C in 1 ml mixture which contained 50 μM imidazole, 20 μM $MgCl_2$, 100 μM $NH_2OH \cdot HCl$, 10 μM ATP, 25 μM 2-mercaptoethanol, 2.5–20 μM L-glutamate and 0–60 μM of inhibitor. Final pH 7.2. Reaction time was 15 min. K_i values were determined from double reciprocal plots of velocity against glutamate concentration.

All analogues listed in the table, although structurally quite diverse, are effective inhibitors of glutamine synthetase, acting competitively against L-glutamate.

The inhibition constants K_i shown in the table indicate that all the analogues examined have substantial affinities to rat liver glutamine synthetase. In fact, the values of K_i obtained for most of our analogues are of the same order of magnitude as K_M for L-glutamate and in four cases (compounds 1, 2, 4 and 6) the inhibitors are bound more tightly than the substrate. This demonstrates that substituting the phosphonic or P-methylphosphinic acid function for the COOH group, regardless of its position in the molecule, is an effective means of producing structural antagonists of glutamic acid. Comparison of inhibition by compounds 1, 3, 5, 7 and 2, 4, 6, 8 shows that the P-methylphosphinic analogues have higher affinities for glutamine synthetase than the compounds with a PO_3H_2 group. Another general trend is that analogues with PO_3H_2 or P(O)(CH$_3$)OH group in γ position (compounds 1–4) are better inhibitors that corresponding α analogues (compounds 5–8).

The effect of an α methyl group is irregular: the K_i values for compounds 1 and 5 are higher than those found for corresponding α-methyl derivatives 3 and 7; note that α-methyl glutamate is not as good substrate for glutamine synthetase as glutamate[14], while the K_i for phosphinothricin (2) is lower than that observed for the α-methyl derivative 4). It is worth noting that compound 4 is a strong inhibitor of rat liver glutamine synthetase, second only to the transition state analogue methionine sulphoximine phosphate[15,16]. Conversion of a COOH group in α or γ phosphonic analogues to an amide or ester function (compounds 9–12) produces inhibitors with K_i values of the same order of magnitude as K_M for L-glutamate. It thus appears that the COOH group is not essential for effective binding by glutamine synthetase.

In view of the efficient inhibition of rat liver glutamine synthetase by phosphonic and P-methylphosphinic substrate analogues reported here it was of interest to examine their effect on other enzymes involved in the metabolism of glutamic acid. Studies performed so far have included *E. coli* glutamate decarboxylase (EC.4.1.1.15), porcine heart alanine and aspartate transaminases (EC.2.6.1.2, EC.2.6.1.1) and bovine liver glutamate dehydrogenase (EC.1.4.1.3). No effect was observed in the case of gluta-

Inhibition of rat liver glutamine synthetase ($K_m = 2.4 \cdot 10^{-3}$ M) by phosphonic analogues of glutamic acid and glutamine

Compound	Structure	K_i [M]
1	$H_2O_3P-CH_2-CH_2-CH(NH_2)-COOH$	$8.8 \cdot 10^{-4}$
2	$HO(CH_3)(O)P-CH_2-CH_2-CH(NH_2)-COOH$	$3.2 \cdot 10^{-4}$
3	$H_2O_3P-CH_2-CH_2-C(NH_2)(CH_3)-COOH$	$6.3 \cdot 10^{-3}$
4	$HO(CH_3)(O)P-CH_2-CH_2-C(NH_2)(CH_3)-COOH$	$5.6 \cdot 10^{-5}$
5	$HOOC-CH_2-CH_2-CH(NH_2)-PO_3H_2$	$1.3 \cdot 10^{-3}$
6	$HOOC-CH_2-CH_2-CH(NH_2)-P(O)(CH_3)OH$	$7.9 \cdot 10^{-4}$
7	$HOOC-CH_2-CH_2-C(NH_2)(CH_3)-PO_3H_2$	$1.6 \cdot 10^{-2}$
8	$HOOC-CH_2-CH_2-C(NH_2)(CH_3)-P(O)(CH_3)OH$	$9.5 \cdot 10^{-3}$
9	$H_2NOC-CH_2-CH_2-CH(NH_2)-PO_3H_2$	$2.5 \cdot 10^{-3}$
10	$H_2O_3P-CH_2-CH_2-CH(NH_2)-CONH_2$	$6.2 \cdot 10^{-3}$
11	$H_2O_3P-CH_2-CH_2-CH(NH_2)-COCH_3$	$8.7 \cdot 10^{-3}$
12	$H_3COOC-CH_2-CH_2-CH(NH_2)-PO_3H_2$	$2.1 \cdot 10^{-3}$

* DATA EXCERPTED FROM THE SUBMITTED REPORT

niate dehydrogenase, while inhibition of decarboxylase and transaminases was insignificant. Thus, recognition of phosphonic and phosphinic analogues as being similar to glutamic acid might be a peculiar property of glutamine synthesizing enzymes. This might be due to the similarity of inhibitors to the intermediate γ-glutamyl phosphate.

1 This study was supported by grant R.I.9.
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Reviewed by: Whang Phang, Ph.D. Whang Phang
Secondary Reviewer: Marcia van Gemert, Ph.D. M. van Gemert 3/15/88
Section III, Tox. Branch (TS-769c)

DATA EVALUATION REPORT 006936

STUDY TYPE: Inhibition of glutamate decarboxylase by phosphonic analogues of glutamic acid

CHEMICAL: phosphonic analogues of glutamic acid (for structures, see the attached article)

EPA ACCESSION No: 403456-29 CASWELL No.: 580I

EPA ID No: 8340-EO/8340-EI EPA PROJECT No: 8-0146

SPONSOR: Published study in Experientia 41: 643-644 (1985) (Attachment)

TESTING LABORATORY: Universite de Bordeaux II, France

CITATION: Lacoste, A. M., Mansour, S., Cassaigne, A. & Neuzil, E. (1985). Effect of phosphonic analogues of glutamic acid on glutamate decarboxylase. Universite de Bordeaux II, France; Report No.: A34389; April 17, 1984. Submitted by Hoechst Celanese Corp., Sommerville, NJ. Aug 27, 1987.

SUMMARY: The relevant results indicate that 2-amino-4-(methylphosphino)butyric acid, which is structurally similar to Hoe 039866, inhibited mammalian brain glutamate decarboxylase. However, the report is a published article, and the results can not be verified. The study is Unacceptable.

A34389
1(2)

Experientia 41 (1985), Birkhäuser Verlag, CH-4010 Basel/Switzerland

Effect of phosphonic analogues of glutamic acid on glutamate decarboxylase[1] *

006936

A.M. Lacoste, S. Mansour, A. Cassaigne and E. Neuzil

Laboratoire de Biochimie médicale, Université de Bordeaux II, F-33076 Bordeaux Cedex (France), 17 April 1984

Summary. Among the phosphonic analogues of glutamic acid, only 4-amino-4-phosphono butyric acid, the compound which shows the highest affinity for pyridoxal phosphate, inhibits competitively both *Escherichia coli* and rat brain glutamate decarboxylases. Phosphinothricin, 2-amino-4-(methylphosphino)butyric acid, is a strong inhibitor of the mammalian enzyme.

Key words. *E. coli*, rat brain; glutamate, phosphonic analogues; glutamate decarboxylase; pyridoxal phosphate.

The phosphonic analogues of α-aminocarboxylic acids show a strong affinity for pyridoxal 5'-phosphate[2] (PLP); some of these analogues behave as antagonists in metabolic reactions involving PLP-dependent enzymes. A typical example is the inhibitory activity of 1-amino-ethylphosphonic acid on alanine racemase[3], knowledge of which promoted the synthesis of the new antibiotic alafosfalin[4], another example is the potent inhibition of rat liver tyrosine transaminase by the phosphonic analogue of tyrosine[5]. Glutamate decarboxylase (GAD, EC 4.1.1.15) is a widely distributed pyridoxal-phosphate protein which catalyzes the synthesis of 4-aminobutyric acid. The bacterial enzyme has a high substrate specificity, practically limited to L-glutamate and L-glutamine[6], whereas the brain enzyme also acts on L-cys L-cysteine sulphinate and L-aspartate[7]. We have investigated the effect of the three phosphonic analogues of glutamic acid (table) and of some related compounds on bacterial (*Escherichia coli*) and mammalian (rat brain) glutamate decarboxylases and on free pyridoxal phosphate.

The formation of a Schiff base between the amino group of the various analogues and the formyl group of PLP was measured using a spectrophotometric method, as described earlier[8]. The apparent dissociation constants K_a listed in the apparent dissociation constants K_a listed in the table:

$$K_a = \frac{[\text{PLP}][\text{amino acid}]}{[\text{aldimine}]}$$

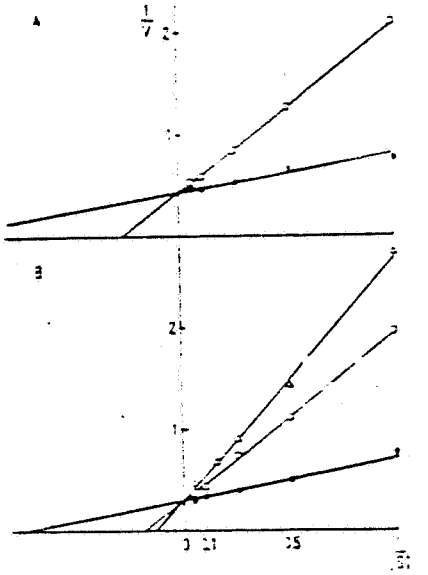
confirm the results obtained in our previous paper with other molecules. Compound 2, the α phosphonic analogue of glutamic acid, reacts with PLP at a faster rate than the dicarboxylic compound; this behavior may be related to the differences of $pK_{NH_3^+}$ existing between the two series of amino acids[9]. On the other hand, when a phosphonic group replaces the γ-COOH of glutamic acid, giving compound 3, the affinity for PLP is greatly diminished; this unfavorable effect of $-PO_3H_2$ in the γ position was also observed with compound 4, since this diphosphonate gives a K_a ranging between the values determined for glutamic acid and compound 2; the presence at the end of the molecule of a strongly acidic group probably induces conformational changes of the carbon chain resulting in a lowered reactivity of the amino group. Compounds 2 and 4, which showed a higher affinity for PLP than compound 1, may then be expected to inhibit glutamate decarboxylase.

The enzymatic activity of *E. coli* glutamate decarboxylase (Sigma Chem. Co. St.Louis, Mo. USA) and of a partially purified rat brain enzyme[8] was measured by determining the rate of release of $^{14}CO_2$ from 1-^{14}C-L-glutamate (Amersham), using a technique slightly modified from Fonda[8]. Assays, in duplicate, were carried out at 30 °C in small conical plastic Eppendorf tubes containing the enzyme solution, PLP ($5 \cdot 10^{-4}$ M), and the potential inhibitor (0–30 mM) in a final volume (50 µl) of buffer (0.1 M pyridine-HCl, pH 4.22 for the bacterial enzyme, 0.1 M phosphate, pH 6.8 for the brain enzyme). A second vessel containing hyamine hydroxide was introduced into the tubes before the reaction was started by adding labeled glutamate (1–20 mM). Blanks without substrate were run in parallel. After an incubation period, CO_2 was finally re-

Affinity of glutamic acid and its phosphonic analogues for pyridoxal 5'-phosphate

Compound	Structure	K_a mM
1	HOOC–CH_2–CH_2–CH(COOH)(NH_2) L-Glutamic acid	6.6
2	HOOC–CH_2–CH_2–CH(PO_3H_2)(NH_2) DL-4-Amino-4-phosphonobutyric acid	2.7
3	H_2O_3P–CH_2–CH_2–CH(COOH)(NH_2) DL-2-Amino-4-phosphonobutyric acid	14.3
4	H_2O_3P–CH_2–CH_2–CH(PO_3H_2)(NH_2) DL-2-Aminopropane-1-diphosphonic acid	4.3

Experimental conditions as described in Cassaigne et al.[2]. Phosphate buffer, pH 8. Compounds 2, 3 and 4 were obtained from Prof. Isbell (Agricultural Univ., College Station, Texas). Compound 3 is obtainable from Calbiochem-Behring, San Diego, Cal.



Competitive inhibition of *Escherichia coli* (A) and rat brain (B) glutamate decarboxylases by 4-amino-4-phosphonobutyric acid (○, 0 mM; ●, 10 mM) and 2-amino-4-(methylphosphinoyl)butyric acid (□, 10 mM; ■, 30 mM). Control curve (●) Si-glutamic acid, mM. The points shown in the figure are the means of three experiments, each one carried out in duplicate.

* STUDY EXCERPTED FROM THE Submitted Report
STUDY #A34389

Experientia 41 (1985), Birkhäuser Verlag, CH-4010 Basel, Switzerland

released by H_2SO_4, trapped with hyamine hydroxide and estimated using a scintillation counter (Intertechnique).

Compound 2, which obviously cannot be a substrate, was the only compound showing inhibitory properties; K_i values obtained through Lineweaver-Burk plots (fig.) were one order of magnitude greater than the K_m of both *E.coli* GAD (K_m = 0.9 mM, K_i = 10 mM) and rat brain GAD (K_m = 1.3 mM; K_i = 10.5 mM). Our results indicate that binding of the 2 phosphonic group at the active site of both enzymes is weakened by steric hindrance.

Compound 3, despite the presence of an α-aminocarboxylic group, was neither a substrate nor an inhibitor. Negative results were also obtained with 4 and with two phosphonic analogues of aspartic acid: 2-amino-3-phosphonopropionic and 2-amino-3-phosphonoprop[illegible]onic acids; the brain enzyme which recognizes L-aspartic acid as a substrate was not inhibited by the two last compounds tested at a concentration 10-fold higher than that of the substrate. Moreover, the inertness of compound 3 may emphasize the importance of the γ-carboxylic group of glutamic acid for enzyme recognition. We have investigated the role of this group by studying the effect on decarboxylase activity of 2-amino-4-(methylphosphino)-butyric acid, also known as phosphinothricin[1], homocysteic acid and norvalin, compounds differing from glutamic acid by the replacement of its γ-COOH group by $-PO(OH)(CH_3)$, $-SO_3H$ and $-CH_3$, respectively. The strict specificity of *E.coli* GAD towards the γ-carboxyl group was confirmed by the lack of inhibitory effect of the above-mentioned substances; this may be related to the recent observation of Vospel et al.[9] that this acidic group is involved in a linkage with an arginine residue in the catalytically active form of *E.coli* GAD. Unlike the bacterial enzyme, brain GAD was able to recognize other acidic groups in the same position: 2-amino-4-(methylphosphino) butyric acid was a potent competitive inhibitor with a K_i of 2.2 mM, a value very close to the K_m for glutamate. L-cysteic and L-homocysteic acids, used in a molar ratio inhibitor/substrate=10, led to inhibition values of 50 and 42% respectively. L-Norvaline was inert towards the two enzymes. It may be emphasized that all the inhibitory molecules tested possess an ω-mono-anionic group like the substrate, a fact which may explain the inertness of 3 and 4 towards brain GAD. However, work in an other field has shown that compound 3 is able to interfere with the neuronal glutamate receptor[10].

The dimethanyl derivative of phosphinothricine is a natural compound which exerts its antibiotic properties against several microorganisms through the action of phosphinothricin on glutamine synthetase[4]; phosphinothricin is also a potent inhibitor of rat liver glutamine synthetase[12]. We have shown here another target site of this compound in eucaryotic enzymatic system.

1 We acknowledge the gift of a sample of phosphinothricin by Prof. Przemyslaw Mastalerz as well as the technical assistance of Denise

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Reviewed by: Whang Phang, Ph.D. 0/16/88
Secondary Reviewer: Marcia van Gemert, Ph.D. 8/17/88
Section III, Tox. Branch (TS-769c)

DATA EVALUATION REPORT

006936

STUDY TYPE: Contact hypersensitivity study (guinea pigs)

CHEMICAL: HOE 039866; monoammonium [2-amino-4-(hydroxymethyl-phosphinyl)butanoate]; Ignite® (Formulation)

EPA ACCESSION No: 403456-03 CASWELL No.: 580I

EPA ID No: 8340-EO/8340-EI EPA PROJECT No: 8-0146

SPONSOR: Hoechst Celanese Corp.

TESTING LABORATORY: RCC, Research & Consulting Co. AG, P.O. Box CH 4452; Itingen, Switzerland

CITATION: Ullmann, L. and Sachsse, K. (1985). Contact Hypersensitivity to Hoe 039866 OH SL 18 A505 in albino guinea pigs, Buehler test. RCC, Research & Consulting Co. AG, Switzerland; Report No.: A32847; Project No.: 053010. Nov 18, 1985. Submitted by Hoechst Celanese Corp., Sommerville, NJ. Aug 27, 1987.

SUMMARY: The test agent did not cause skin hypersensitivity, and the study is classified as Minimum (for end use product).

MATERIALS AND METHODS:

Test article: Hoe 039866 OH SL 18 A505; liquid formulation 200 g/L. The test article reported to be a blue green liquid with 18.2% (w/v) purity.

Animals: 8-9 weeks old Dunkin-Hartley albino guinea pigs weighing 326-414 for males and 354-456 g for females.

Experimental procedures: The details of the procedures are excerpted from the submitted report and presented in the Appendix. In summary, groups of 10 animals/sex were applied with 50% of the test article on the shaved skin 3 times/week (wk) for 3 wks and the animals were kept for 2 weeks without treatment. Subsequently, the animals were challenged with the test article for 6 hrs. Twenty-four to 48 hrs later, the treated skin site was graded on a scale of 0 (no erythema) to 3 (marked erythema).

A second challenge was applied 2 wks after the first, and similar observational procedures were used as the first challenge.

Five guinea pigs/sex were used as the vehicle controls (water) and tested in a similar manner as the treated animals.

593

RESULTS:

At day 1 and day 3 of the test, shortly after removal of the test material, the test article-treated animals showed severe sedation and dyspnea.

Increased incidence of the skin hypersensitivity was not observed. The necropsy findings did not reveal any compound related effects.

Results of a previous study using the same strian of test animals as the present study showed that the positive control (DNCB) caused marked skin hypersensitivity.

DISCUSSION:

The test agent did not cause skin hypersensitivity under current testing conditions. The study is classified as Minimum (for end-use product).

APPENDIX

Information excerpted from the submitted report

(HOECHST REPORT No.: A32847)

RIN 5218-93 / Tox Review for Glufosinate Log # 293

Page_____ is not included in this copy.

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____ Identity of product inert ingredients.

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____ Description of the product manufacturing process.

____ Description of quality control procedures.

____ Identity of the source of product ingredients.

____ Sales or other commercial/financial information.

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____ The product confidential statement of formula.

____ Information about a pending registration action.

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Reviewed by: Whang Phang, Ph.D. 9/19/88
Secondary Reviewer: Marcia van Gemert, Ph.D. 9/19/88
William Sette, Ph.D. 9/19/88
Section III, Tox. Branch (TS-769c)

DATA EVALUATION REPORT 006936

STUDY TYPE: Observational assessment of neurotoxicity and determination of glutamine synthetase (GS) activity, NH_4^+ and glutamate levels in brain, liver, and kidney of female Wistar rats

CHEMICAL: HOE 039866; monoammonium [2-amino-4-(hydroxymethylphosphinyl)butanoate]; Ignite®

EPA ACCESSION No: 403456-25 CASWELL No.: 580I

EPA ID No: 8340-EO/8340-EI EPA PROJECT No: 8-0146

SPONSOR: Hoechst Celanese Corp.

TESTING LABORATORY: Hoechst AG, 6230 Frankfurt AM Main 80
Federal Republic of Germany

CITATION: Ebert, E., Mayer, D., Leist, H. -K., and Langer, K. -H. Hoe 039866-active ingredient technical (Code: Hoe 039866 OH ZC95 0001)- Examination of the mode of action following a single oral dose in female Wistar rats; determination of glutamine synthetase activity and of the NH_4^+ and glutamate levels in various organs. Hoeschst AG: Report No.: A34242; Project No.: 86.1003. Sept 2, 1986. Submitted by Hoechst Celanese Corp., Sommerville, NJ. Aug 27, 1987.

METHODS AND MATERIALS:

1). Test compound: Hoe 039866, technical 96.9% purity; crystalline white powder; code: Hoe 039866 OH ZD97 0001

2). Animal: Female Wistar rats were obtained from Hoechst AG, Kastengrund (SPF breeding colony). The animals were 8-9 weeks old with a mean body weight of 182 gm.

3). Experimental procedures: Based upon the results of an acute oral toxicity study, which yielded an LD_{50} of 1620 mg/kg, the animals were randomly assigned to various test groups as follows:

Group	Dose mg/kg	No. of animals	Sacrifice No. of days after treatment 1	3	7.5
1(Con)	0	15	5	5	5
2(Low)	200	15	5	5	5
3(Mid)	300	15	5	5	5
4(High)	1600	30	10	10	10

The test compound was dissolved in water and administered to the above groups of animals by gavage. After treatment, the rats were observed for signs of neurotoxicity according to the method of S. Irwin (Psychopharmacologia 13, 222-257, 1968). The examination procedures and scoring system for functional observations were presented in Appendix 1. According to the report, the functional observations for neurotoxicity were conducted as follows:

a). Animals sacrificed on day 3 were observed 4 to 8 hrs after treatment and subsequently once daily.

b). Animals sacrificed on day 7.5 were observed 12 to 16 hrs after treatment and subsequently once daily.

On days 1, 3, and 7.5 after treatment, the appropriate groups of animals were weighed and sacrificed. Gross examinations were carried out. Liver, kidneys, and brain were removed, weighed, and frozen. Subsequently, the levels of glutamine synthetase (GS) activity, NH_4^+, and glutamate were determined in group 1 and group 4 animals, which showed compound-related changes. The analytical methods were presented in Appendix 2. The level of brain acetylcholinesterase (AchE) activity was measured, but no analytical procedures for measuring AchE were included in the report.

4). Statistical methods used in this study are presented in Appendix 3.

RESULTS:

1). Mortality: 3 animals from 1600 mg/kg group died within 3 days after treatment. All others survived to the scheduled sacrifice.

2). Clinical signs: The clinical signs as described in the submitted report (Hoechst Report No. A34242; p 14) are excerpted and presented below:

" Clinical signs in the 200 mg/kg group were enhanced spontaneous activity and isolated pilo-erection, but these were confined to day 1 after treatment.
The animals in the 800 mg/kg group exhibited rather frequent enhancement of spontaneous activity, and in one case convulsions and Straub tail, from 12 to 24 hours after treatment.
In the animals of the highest dose group (1600 mg/kg body weight) isolated instances of diarrhoea were observed, starting 6 hours after treatment, and one animal also exhibited convulsions after handling; enhancement of spontaneous activity and pilo-erection also occurred fairly frequently 12 hours after treatment. Intoxication reached a maximum on days 2 and 3 after treatment, and was manifested additionally in tono-clonic convulsions, squatting position, contracted flanks, lagophthalmos, drowsiness, reduced respiratory rate, and blood-incrusted eyelids and mouth. "

3). Functional observation:

The detailed results for functional observations for the test animals are excerpted from the submitted report (Hoechst Report No. A34242) and presented in Appendix 4.

In summary, 200 and 800 mg/kg female rats showed some behavioral impairments as indicated by reduced reactions (Table 1b & 1c). These deficits began to recede at day 5.

Animals in 1600 mg/kg groups showed marked CNS excitability at 1 day after treatment. Some animals in this dose level showed convulsions and spasms which were followed by exhaustions. These signs began to recede at day 3 (Tables 1d & 1e).

4). Glutamine synthetase (GS) activity (Table 2):

Brain: Relative to the controls decreases in brain GS activity were observed in all dosed rats. The decrease in 800 mg/kg was more than 10%, and that in 1600 mg/kg group was significantly different from control value (Table 2). These decreases also appeared to show a dose-related response at all examination intervals.

Liver: The liver GS levels in all treated animals were markedly decreased, and these decreases were statistically significant (Table 2).

Kidney: At day 1 after treatment there were statistically significant decreases in kidney GS levels in all treated animals. At day 3, decreases were seen in all dose animals, but only those in 800 and 1600 mg/kg animals showed statistical significance. At day 7.5, the kidney GS levels in treated and control animals were comparable (Table 2).

5). NH_4^+ level in various organs (Table 3):

Brain: Variable results were obtained for NH_4^+ levels at different examination times (Table 3). At day 1, a lower mg was observed in all treated animals relative to the controls, and the decrease in 800 and 1600 mg/kg groups was statistically significant. At day 3, the values of treated and control animals were comparable. In contrast, at day 7.5, there was an increase in all treated animals. Although this increase was slight, it was statistically significant for the 800 and 1600 mg/kg animals.

Liver: There was an increase of liver NH_4^+ levels at day 1, a decrease at day 3, and practically no changes at day 7.5 in 1600 mg/kg rats relative to those of the controls. The

NH_4^+ level in other dose groups were comparable to that of the controls (Table 3).
Kidney: There was a decrease in kidney NH_4^+ level in all treated animals at day 1, and that of 200 and 1600 mg/kg groups showed statistical significance. At other examination periods the levels were comparable between treated and control animals (Table 3).

6). Glutamate levels in various organs (Table 4):

Brain: At all examination times, brain glutamate level was reduced in 800 and 1600 mg/kg animals. At 7.5 day, statistically significant lowering of glutamate level was observed in 800 and 1600 mg/kg rats relative to that of the controls.

Liver: A statistically significant increase in liver glutamate level of 1600 mg/kg animals was observed in days 1 and 3 (Table 4).

Kidney: Kidney glutamate levels were elevated in all treated animals relative to that of the controls in days 1 and 3. The increase in 200 mg/kg rats was statistically significant on day 1; in 800 mg/kg, on days 1 and 3. On day 7.5, glutamate levels of treated and control animals were comparable (Table 4).

7). Brain AchE activity: Inconsistent changes in brain AchE were found at various examination intervals. At days 1 and 3, there were some small increases and decreases among different treated groups, and at day 7.5, there was a decrease in brain AchE among all treatment groups (Table 5). The drop in brain AchE in high dose animals at day 7.5 was statistically significant. The individual animal data indicated that 5/17 high dose animals had decreased brain AchE activity, but these five animals did not show any clinical signs which were indicative of AchE inhibition.

8). Body weights and organ weights: Body weights and organ weights of treated and control animals were comparable. The mean body weights are presented in Table 6, and a representative set of organ weight data are presented in Table 7.

TABLE 6†
Body Weights (gm) of Control and Treated Female Rats

Examination Interval (day)	Doses (mg/kg) 0	200	800	1600
1	198±12	189± 7	181±16	173±18
3	195± 9	209±12	191±18	178±28
7.5	196±18	203±10	208±15	193±32

006936

† Data excerpted from submitted report (Hoechst report No. A34242)

TABLE 7†

Representative Organ Weights (gm) of Control and Treated Female Rats At Examination Day 7.5

Doses(mg/kg)	Liver	Kidney	Brain
0	6.98±1.00	1.33±0.14	1.77±0.05
200	7.51±0.62	1.28±0.06	1.82±0.21
800	7.98±0.76	1.38±0.16	1.80±0.07
1600	7.43±1.62	1.30±0.08	1.77±0.15

† Data excerpted from the submitted report (Hoechst Report No. A34242)

DISCUSSION and CONCLUSION:

In an earlier study, groups of female Wistar rats were orally administered a single dose of Hoe 039866 (200 or 800 mg/kg), and the animals were sacrificed 4 hrs after dosing. The levels of GS activity, NH_4^+, glutamine, and glutamate in different organs were determined; no signs of neurotoxicity were reported. However, functional observations for any signs of neurotoxicity were not conducted (Hoechst Report No. A34503; EPA Accession No. 403456-23). As a result, an additional study was conducted at a high dose and for longer observation times after treatment.

For the present study, groups of females Wistar rats were orally administered the test agent at doses of 200, 800, and 1600 mg/kg. The test animals were observed daily for any neurotoxicity signs prior to sacrifice. Five rats/dose or 10 rats of high dose group were sacrificed on days 1, 3, and 7.5. The levels of GS activity, NH_4^+, and glutamate in brain, liver, and kidneys were determined. In addition, body weights and organ weights were measured. The following compound-related effects were found.

1). Clinical signs: 200 and 800 mg/kg animals showed signs of increased spontaneous activity. In 1600 mg/kg animals, there were insolated incidences of diarrhea, increased spontaneous activity, piloerection, and tono-clonic convulsion.

2). Functional observation for neurotoxicity signs: In 200 and 800 mg/kg rats, there were signs of behavioral impairments as indicated by decreased reactions. In 1600 mg/kg animals, marked CNS excitability as indicated by convulsion, spasms, and exhaustion were noted.

3). GS activity:

Brain: GS activity was decreased in 800 and 1600 mg/kg rats.

Liver: GS level in liver was markedly decreased in all dosed animals at all examination intervals.

Kidney: At examination days 1 and 3, there were decreases in kidney GS levels in all treated animals, but at day 7, the GS levels practically returned to the level of the control animals.

4). Glutamate level:

Brain: Brain glutamate levels were decreased in 800 and 1600 mg/kg animals at all examination intervals.

Liver: Increases were observed in in 1600 mg/kg rats at days 1 and 3 examination intervals.

Kidney: At days 1 and 3 examination intervals, there were increases in kidney glutamate levels in all dose groups; however, at day 7.5, the levels approached that of the controls.

5). NH_4^+: The NH_4^+ levels in various organs, at different times, and in three dose groups were variable. The biological significance of the inconsistent results for NH_4^+ was doubtful.

The compound-related findings of behavioral impairment, increased spontaneous activity, and changes in GS activity levels in kidney and liver at lowest dose (200 mg/kg) did not allow an NOEL to be established. This study used only females rats, and no explanation was given concerning this selective experimental condition. In addition, no functional observation data were presented for treated animals which were sacrificed on day 1 after treatment (specifically animals in cages 2 to 5). It was also hard to tell how many animals showed which responses from the combined scores (e.g. -0.8).

This study is classified as Supplementary.

RIN 5218-93 / Tox Review for Glufosinate Log #293

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____ Description of the product manufacturing process.

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Reviewed by: Whang Phang, Ph.D. 8/2/88
Secondary Reviewer: Marcia van Gemert, Ph.D. 8/4/88
Section III, Tox. Branch (TS-769c)

DATA EVALUATION REPORT (DER)

006936

STUDY TYPE: Neurotoxicity study in white Leghorn hens.

CHEMICAL: Monoammonium [2-amino-4-(hydroxymethylphosphinyl)-butanoate]; Hoe 039866 liquid concentrate 40; Ignite®

EPA ACCESSION No: 403456-04 CASWELL No.: 580I

EPA ID No: 8340-EO/8340-EI EPA PROJECT No: 8-0146

SPONSOR: Hoechst Celanese Corp.

TESTING LABORATORY: Hoechst AG, 6230 Frankfurt AM Main 80
Federal Republic of Germany

CITATION: Leist and Weigand (1979). Neurotoxicity study with Leghorn-hens Hoe 039833 OH RH012 (liquid concentrate 40). Hoechst AG; Report No.: A21969; Project No.: 275/79; Nov 29, 1979. Submitted by Hoechst Celanese Corp., Sommerville, NJ. Aug 27, 1987.

SUMMARY: Oral administration (gavage) of Hoe 039866 liquid concentrate 40 at doses ranging from 1,600 mg/kg to 10,000 mg/kg did not cause acute toxicity in white Leghorn hens. In the neurotoxicity study, the hens were dosed with 10,000 mg/kg, no abnormal behavior or histological changes in the brain, spinal cord, and sciatic nerve were seen.

The study has many deficiencies as indicated in the Discussion section of this DER, and it is classified as Supplementary.

METHODS AND MATERIALS:

Test chemical: Hoe 039866 liquid concentrate 40 (Code: Hoe 039866 OH RH012); colorless liquid; purity not specified.

Animals: White Leghorn-hens weighing approximately 1.5 kg were obtained from Horst Halt, 6479 Forsthaus Finkenlock, Post uber Nidda. These birds were 6-7 months old egg laying hens.

Experimental procedures:

Acute Toxicity: 2 hens/dose were gavaged with 1600, 2500, 4000, 5000, 6300, and 10000 mg/kg of Hoe 039866 and observed for 14 day.

Neurotoxicity: The hens were divided into 4 groups (5 hens/group), and treatments (gavage) were carried out as follows:

Group	No. of Hens	Treatment
1	5	10,000 mg/kg Hoe 039866
2	5	test substance + antidote
3	5	TOCP Positive control
4	5	negative control

TOCP: triorthocresyl phosphate (500 mg/kg)(5.75% in sesame oil)
Negative control: 8.7 ml/kg sesame oil
antidote: 10 mg/kg atropine and 4 mg/kg Toxogonin (i.v.)

A secondary treatment was carried out at 21 days after the primary treatment as the following:

Group 1: 10,000 mg/kg Hoe 039866 (gavage)
2: antidote = 10 mg/kg atropin and 4 mg/kg toxogonin (i.v.)
3: no further treatment
4: 8.7 mg/kg saline solution (gavage)

The birds were examined for abnormal behavior and health status daily, and the body weights were measured twice weekly. At 42 days, the survival birds were sacrificed and sciatic nerves, spinal cord, and brain were "perfused" with 8% formalin. Histological examinations were carried out on these tissues.

RESULTS:

Acute toxicity: A single oral administration of the test agent at doses up to 10,000 mg/kg did not produced characteristic neurotoxicity signs as that produced by TOCP. At 10,000 mg/kg the birds showed "marked shaking of the head during the first 5 days" following dosing. No effect on body weights was observed. Death was only seen in 1/2 birds of 2,500 mg/kg group. This bird was reported to die from being severely pecked by other birds on the anal region.

Neurotoxicity:

Positive controls (TOCP): Increased tendencies of mutual anal pecking was observed. Decreased body weights and diarrhea were found in this group of birds. It should be noted the report did not contain any data on body weight or individual animal data on clinical observation.

Histological examinaton of the nervous system of the positive control animals showed that demyelinization and marked glial cell proliferations occurred in cerebellar peduncles. In spinal cord, glial cell foci and swollen axons were seen.

Test chemical treated hens: No death occured in this group during the test. Diarrhea was observed in some animals. Histological examination on brain, spianl cord, and sciatic nerves did not show any lesions which could be attributed to the test agent.

DISCUSSION:

The data of this study indicated that Hoe 039866 liquid concentrate 40 did not cause acute toxicity or neurotoxicity in white Leghorn hens.

Although the study provides useful information, it has several deficiencies which include lack of individual animal data on clinical observation and no information on purity of the test chemical. The test chemical was a formulation instead of technical grade. In addition, the protocol of the neurotoxicity study has not clearly indicated the length of treatment. The study is, therefore, classified as Supplementary.