3/14/84 DB-1399 TR-3682

003682

Caswell No(s).: 431 C

To: Mountfort acc nos. 071786-071795 + 07185

Registration No(s).:

Pesticide Petition No(s).: (1) 8340-EUPT, (2) 3G2940

Chemical(s): Fenoxaprop-ethyl

Requested Action(s): EUP and temporary tolerance for use on soybeans

Recommendation: Request is supported toxicologically

Inert(s) cleared 180.1001: yes

% of ADI occupied: Existing: new chemical Resulting:

Resulting % increase in TMRC:

Data considered in setting the ADI:

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Attached (?): ADI printout: ~~YES~~/NO; TOX "one-liner": YES/~~NO~~; DER: YES/~~NO~~

Existing regulatory actions against registration:

RPAR status:

New Data: See reviews attached.

Comments: See conclusions on 90 day rat study.

~~Data gaps:~~ Also see comments on micronucleus test. An acceptable chromosome aberration analysis in vivo or in vitro, and a gene ~~Remarks:~~ mutation in mammalian cells in culture will be needed for registration. See attached comments on mutagenicity.

Reviewer: W Thomas Edwards Date:

Section Head: William W. Guthrie 3/9/84 Branch Chief: [signature] 3/14/84

1/93

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Comments on fenoxaprop-ethyl metabolism studies.

The metabolism studies to support evaluation of this new chemical varied from EPA guideline procedures in several important respects. These studies are useful but more information is needed for registration.

Each study was performed at only one dose level. The dose levels for the two studies were different (about 2 mg/kg and 40 mg/kg) other parameters are to be found in the attached current EPA guidelines.

Also it would be helpful to follow metabolite distribution-storage (at zero) and distribution with the other ring tagged.

Series 85: SPECIAL S[illegible]

(NOTE: Section 85-1 of this [illegible] prepared in conformity with the guidelines developed by the [illegible] Development. Those guidelines [illegible] requirements of this section under [illegible]

Section 85-2 is essentially [illegible] guidelines proposed on August 22, 197[illegible]. Public comments on this section have not yet been used to [illegible]. When the revision is prepared, it will replace the current [illegible]

§ 85-1 Metabolism study.

(a) When required. Data from a general metabolism study are required to support the registration of each manufacturing-use product and end-use product which requires a chronic toxicity study or an oncogenicity study, in accordance with 40 CFR § 158.135.

(1) See, specifically, 40 CFR § 158.50 and § 158.135 to determine whether these data must be submitted. Section II-A of this subdivision contains an additional discussion of the "Formulators' Exemption" and who must submit the required data as a general rule.

(b) Purpose. (1) Data from studies on the absorption, distribution, excretion, and metabolism of a test chemical are desirable [illegible] evaluation of test results from other toxicology studies and [illegible] polation of data from animals to man. Such studies should [illegible] on each chemical of toxicological concern. The concern may be predicated on the level and type of toxicity observed (or anticipated) and by the magnitude of potential human exposure to the chemical. Flexibility is needed in the conduct of metabolism studies and depends on the characteristics of the test chemical being investigated. The main purpose of metabolism studies it to produce data which fortify the understanding of the safety of the chemical in consideration of its intended uses and anticipated human exposure.

(2) In addition to the general reasons stated in paragraph (b)(1) of this section, a metabolism study may be performed for the following purposes:

(i) To determine the amount and rate of absorption of the test chemical at different dose levels;

(ii) To determine the pattern of distribution of the test chemical among tissues, organs, and fluid compartments at different dose levels, after single and repeated dosages;

(iii) To identify and, to the extent possible, quantify significant metabolites;

[illegible]

[illegible] biotransformation of [illegible] and/or metabolites [illegible]

[illegible] absorption, [illegible] and distribution [illegible] of several doses [illegible] metabo- [illegible] define [illegible]

[illegible] (1) [illegible] shall be per- form[illegible] ingredient, usually in an [illegible]-labeled [illegible] radioactive, such as ^{14}C, [illegible] and [illegible], or [illegible] and [illegible]. In some cases, more than one label per [illegible] advantageous. Labels shall be placed in positions [illegible] the "core" of the molecule or significa[illegible], one should avoid placing ^{14}C in positions [illegible] be [illegible] to enter the carbon pool of the test animal[illegible] exchangeable labeling shall be avoided.

(2) [illegible] may not be required if sufficiently selective and sensitive physical [illegible] tests for identifying the compound and its metabolites are used.

(3) Some animals are to receive repetitive doses of nonlabeled chemical substance (analytical grade).

(d) Test procedure. (1) Choice of method. A registrant may, after consultation with the Agency, utilize a modified or completely different experimental design if it provides the information required by this section.

(2) Animal selection. (i) Species and strain. The preferred species is the rat. If another mammalian species is used, the tester should provide justification/reasoning for its selection. Commonly-used laboratory strains should be employed. Preliminary studies may be performed in several species to develop information on comparative metabolism. Information derived from preliminary studies may help in the selection of species for subsequent toxicity tests.

(ii) Age. Young adult animals should be used. For specific purposes, a comparative study using very young or very old animals may provide information about the effects of age on the metabolism.

(iii) Sex. (A) Equal numbers of animals of each sex should be used at each dose level.

(B) Females should be nulliparous and nonpregnant.

(iv) Numbers. At least ten animals (five females and five males) should be used at each dose level.

(3) Dose levels and dose selection. (i) At least two dose levels should be used.

(ii) The low dose should correspond to a no-effect level.

(iii) The upper dose should produce toxic or pharmacologic signs, but should not produce severe effects or a high incidence of mortality which would prevent a meaningful evaluation.

(iv) The determination of absorption, tissue distribution, and elimination should be studied as a function of single or repeated doses.

(4) Observation period. Animals should be kept in individual metabolism cages for 7 days after radioactive dose or until 90+ percent of the administered dose is excreted (whichever occurs first), at which time all of the animals should be killed.

(5) Administration of the test substance. (i) Route of administration. The study should be done using the oral route (capsule or gavage). If another route of administration is used, the tester should provide justification/reasoning for its selection. When vehicles are used, attention must be given to the possibility that they may interfere with the kinetics of the test chemical.

(ii) Animal groups. The following four groups of animals should be studied:

(A) Group A. These animals should each receive a single intravenous dose of the labeled test substance at the low dose. If it is not possible to dissolve the test substance in physiological saline or water, this group may be omitted.

(B) Group B. These animals should each receive a single oral dose (by capsule or intubation) of the labeled test substance at the low dose.

(C) Group C. These animals should each receive a series of single daily oral doses of the nonlabeled test substance (by capsule or intubation) over a period of least 14 days, followed at 24 hours after the last dose by a single oral dose (by capsule or intubation) of the labeled test substance. Each dose should be the low dose level.

(D) Group D. These animals should each receive a single oral dose (by capsule or intubation) of the labeled test substance at the high dose level.

(6) Observations of animals. (i) Distribution. For all animals in groups B, C, and D, the quantity of label in tissues and organs should be measured at sacrifice by suitable methods with particular attention to bone, brain, fat, gonads, heart, kidney, liver, lungs, muscle, spleen, tissues which displayed pathology (in this or prior studies), and residual carcass.

(ii) Metabolism. Urine and feces from all groups should be analyzed by suitable methods in order to determine the extent of absorption and

biotransformation and to identify the metabolites. An assay method for detection of each major metabolite may be requested by the Agency.

(iii) Excretion. Quantities of label in urine, feces, and expired air should be measured at appropriate intervals (e.g., 4, 8, 12, and 24 hours, 1.5, 2, 3, 4, 5, 6, and 7 days) throughout the study for all animals. However, if a preliminary study shows no volatile label materials are exhaled during the period of zero to 24 hours after dosing, such evidence may be submitted in lieu of measuring label in the expired air for this study.

(e) Data and reporting.

(1) Treatment of results. Data shall be summarized in tabular form.

(2) Evaluation of results. Results, where appropriate, shall be evaluated statistically.

(3) Test report. In addition to the information required by § 80-4, the test report shall include the following data derived from tests on animals in all groups:

(i) Quantity of isotype, together with percent recovery of the administered dose, in feces, urine, and the following tissues and organs of animals in all groups:

(A) Bone;

(B) Brain;

(C) Fat;

(D) Testes;

(E) Heart;

(F) Kidney;

(G) Liver;

(H) Lung;

(I) Blood;

(J) Muscle;

(K) Spleen;

(L) Tissues which displayed pathology (in this or prior studies);

(M) Literus; and

(N) Residual carcass.

(ii) Percent absorption; if possible, percent absorption by the oral route in Groups B, C, and D;

(iii) A full description of the sensitivity and precision of all procedures used to produce the data;

(iv) Information on the degree (i.e., specific activity for a radiolabel) and site(s) of labeling of the test substance; and

(v) Counting efficacy data; such data should be recommended, however, only upon specific request of the Agency.

(f) Additional metabolism studies. Additional, more specific studies may be required to clarify important points.

(1) Some areas for possible further study include:

(i) Identification of tissue residues;

(ii) Binding by macromolecules in the blood, liver, gonads, and other tissues; plasma binding studies may be conducted, usually in vitro with plasma;

(iii) Placental transfer; placental transfer of a chemical substance may be determined by dosing pregnant rodents with chemicals and assaying their fetuses for the chemical;

(iv) Entrance into breast milk;

(v) Biotransformation by specific organs, tissues, and cell fractions; and

(vi) Absorption by dermal or inhalation routes of exposure.

(2) Additional species may be utilized, since the rat and dog differ significantly in metabolic pattern.

§ 85-2 Domestic animal safety testing.

(a) When required. Data from tests on domestic animals may be required in accordance with 40 CFR § 158.135 to support the registration of an end-use product if cats, dogs, cattle, pigs, sheep, or other domesticated animals will be exposed to the pesticide product, including, but not

limited to, exposure through direct application for pest control and consumption of treated feed. The applicant for registration should consult with the Agency to determine what toxicological data are required. In some cases, the data resulting from studies performed on laboratory and nondomestic animals can be extrapolated to the domestic species likely to be exposed. In these cases, no additional testing will be required.

(b) Testing. Data from any of the studies described in this subdivision may be required, including, but not limited to, the following:

(1) Acute oral toxicity;

(2) Acute dermal toxicity;

(3) Acute inhalation toxicity;

(4) Primary dermal irritation;

(5) Primary eye irritation;

(6) Dermal sensitization;

(7) Subchronic oral dosing;

(8) Cholinesterase inhibition;

(9) Neurotoxicity; and

(10) Teratogenicity.

(c) Standards. Each test should be performed according to the standards specified by the Agency. The applicant should also refer to standards specified in the appropriate sections of this subdivision.

(d) Data reporting and evaluation. (1) The general information required by § 80-4 shall be reported for each test. In addition, each test report shall contain all appropriate data required by the "Data reporting and evaluation" paragraphs of the corresponding sections of this subdivision.

(2) In addition, the applicant should submit any evidence of toxicological effects of the pesticide to domestic animals observed during product performance testing including, in particular, field testing.

§ 85-3 Dermal Absorption Studies of Pesticides. [Reserved]

TOXICOLOGY BRANCH
DATA REVIEW

Study Type: Acute oral toxicity, male rat

Accession Number: 071787 (A:1)

MRID Number:

Sponsor: American Hoechst Corporation

Contracting Lab: Hoechst Aktiengesellschaft, report no. 576/79

Date: October 9, 1979

Test Material: ethyl-2-(4-(6-chlorobenzoxazolyloxy)-phenoxy)-propanoate, 97%, Fenoxaprop-ethyl, technical (Hoe 33171 O H AT203)

Protocol

Test animals were fasted 16 hours before and 2 hours after dosing. A 25% suspension was prepared in sesame oil (25 g/ad 100 ml) and administered once by gavage at various dose levels to male rats as shown in the table below. Animals were observed 14 days following dosing.

Results

"The following mortality rate was recorded in the various dosage groups after termination of the 14-day follow-up period:

Dose mg/kg	Concentration %	Mortalities / Number of animals
1 600	25	0 / 10
2 000	25	0 / 10
2 250	25	4 / 10
2 500	25	7 / 10
5 000	25	10 / 10

Lethally intoxicated animals died between days 1 and 7 after the treatment. The following symptoms were observed: passiveness, disequilibrium, squatting, crawling or crouching, bristled hair, blepharophimosis, seromucous and sanguineous rhinorrhoea."

"The autopsy of the animals that had died produced the following findings: bright spots on the liver, lobular marking of the liver, diffuse reddening of the pancreas. Petechial haemorrhages in the gastric mucosa (fundic part) and in the duodenum, red-black liquid matter in the entire region of the small intestine.

The body weight gains of the surviving animals were normal.

The autopsy of the animals killed after termination of the experiment produced diffuse reddening of all abdominal viscera."

Conclusions

Oral LD_{50} (male rat): 2357(2240-2479)mg/kg bw (p=0.05)

Acute oral toxicity category: III

Core Classification

Guideline

003682

TOXICOLOGY BRANCH
DATA REVIEW

Study Type: Acute oral toxicity, female rats

Accession Number: 071787 (A:2)

MRID Number:

Sponsor: American Hoechst Corporation

Contracting Lab: Hoechst Aktiengesellschaft, report no. 577/79

Date: October 2, 1979

Test Material: ethyl-2-(4-(6-chlorobenzoxazolyloxy)-phenoxy) -propanoate; 97%, Fenoxaprop-ethyl, technical (Hoe 33171 O H AT203)

Protocol

Test animals were fasted 16 hours before and 2 hours after dosing. A 25% suspension was prepared in sesame oil (25 g/ad 100 ml) and administered once by gavage at various dose levels to female rats as shown in the table below. Animals were observed 14 days following dosing.

Results

"The following mortality rate was recorded in the various dosage groups after termination of the 14-day follow-up period:

Dose mg/kg	Concentration %	Mortalities / Number of animals
2 000	25	1 / 10
2 500	25	5 / 10
3 150	25	9 / 10
4 000	25	10 / 10
5 000	25	9 / 10

Lethally intoxicated animals died between days 1 - 4 after dosing. The following symptoms were observed: passiveness, disequilibrium, squatting, crawling or crouching, bristled hair, blepharophimosis, chromodacryorrhoea and increased repiratory rate."

"The autopsy of the animals that had died produced the following macrosopic findings: bright spots on the liver, lobular marking of the liver, diffuse reddening of small intestine and pancreas. Petechial haemorrhages in the gastric mucosa (fundic part) and in the duodenum, uterus reddened, red-black liquid matter in the entire region of the small intestine."

The body weight gains of the surviving animals were normal.

The autopsy of the animals killed after termination of the experiment produced slight lobular marking of the liver."

Conclusions

Oral LD_{50} (female rat): 2500(2230-2780)mg/kg bw

Acute oral toxicity category: III

Core Classification

Guideline

003682

3

TOXICOLOGY BRANCH
DATA REVIEW

Study Type: Acute oral toxicity, male mouse

Accession Number: 071787 (A:3)

MRID Number:

Sponsor: American Hoechst Corporation

Contracting Lab: Hoechst Aktiengesellschaft, report no. 423/79

Date: July 27, 1979

Test Material: ethyl-2-(4-(6-chlorobenzoxazolyloxy)-phenoxy)-propanoate, 97%, Fenoxaprop-ethyl, technical (Hoe 33171 O H AT203)

Protocol

A 25% suspension in sesame oil (25 g/ad 100 ml) was prepared for the acute treatment and administered once by gavage at various dose levels to male mice as shown in the table below. The animals were deprived of food for 2 hours after the treatment.

Surviving animals were observed 14 days following dosing.

Results

"The following mortlity rate was registered in the various dosage groups after termination of 14-day follow-up period:

Dose mg/kg	Concentration %	Mortalities / Number of animals
3 150	25	0 / 10
4 000	25	4 / 10
5 000	25	4 / 10
5 600	25	8 / 10
6 300	25	10 / 10

Lethally intoxicated animals died within day one and five after the treatment. The following clinical symptoms were observed: passiveness, increased respiratory rate, blepharophimosis, disequilibrium, abdominal position, drowsiness, increased lacrimation and jerky respiration. The surviving experimental animals were free from clincal symptoms within 48 or 72 hours after the treatment.

The behaviour and the body weight increments were normal during the follow-up period.

The autopsy of the animals that had died produced the following macroscopic findings: extreme filling of the urinary bladder and marking of the hepatic lobules after doses of 6 300 mg/kg as well as advanced autolysis in all dosage groups.

The macroscopic post-mortem examination of the animals sacrificed after termination of the experiment produced no abnormal findings."

Conclusions

Oral LD_{50} (male mouse): 4670(4180-5130)mg/kg bw (p=0.05)

Core Classification: Minimum

003682

TOXICOLOGY BRANCH
DATA REVIEW

Study Type: Acute oral toxicity, female mouse

Accession Number: 071787 (A:4)

MRID Number:

Sponsor: American Hoechst Corporation

Contracting Lab: Hoechst Aktiengesellschaft, report no. 424/79

Date: July 27, 1979

Test Material: ethyl-2-(4-(6-chlorobenzoxazolyloxy)-phenoxy) -propanoate, 97%, Fenoxaprop-ethyl, technical (Hoe 33171 O H AT203)

Protocol

A 25% suspension in sesame oil (25 g/ad 100 ml) was prepared for the acute treatment and administered once by gavage at various dose levels to male mice as shown in the table below. The animals were deprived of food for 2 hours after the treatment.

Surving amimals were observed 14 days following dosing.

Results:

"The following mortality rate was registered in the various dosage groups after termination of the 14-day follow-up period:

Dose mg/kg	Concentration %	Mortalities / Number of animals
2 500	25	0 / 10
3 150	25	0 / 10
4 000	25	0 / 10
5 000	25	3 / 10
5 600	25	8 / 10
6 300	25	6 / 10

Lethally intoxicated animals died within 1 to 7 days after the treatment. The following clinical symptoms were observed: passiveness, blepharophimosis, increased respiratory rate, disequilibrium, abdominal position, drowsiness, increased lacrimation and jerky respiration. The surviving experimental animals were free from clinical symptoms within 48 or 72 hours after dosing.

The behaviour and the body weight increments were normal during the follow-up period.

The macroscopic post-mortem examination of the animals that had died or were sacrificed after termination of the experiment produced no conspicious findings."

Conclusions

LD_{50} (female mouse): 5490(5010-6140)mg/kg bw (p=0.05)

Core Classification: Minimum

TOXICOLOGY BRANCH
DATA REVIEW

Study Type: Acute oral toxicity, dog

Accession Number: 071787 (A:5)

MRID Number:

Sponsor: American Hoechst Corporation

Contracting Lab: Hoechst Aktiengesellschaft, report no. 403/79

Date: July 11, 1979

Test Material: ethyl-2-(4-(6-chlorobenzoxazolyloxy)-phenoxy) -propanoate, 97%, Fenoxaprop-ethyl, technical (Hoe 33171 O H AT203)

Protocol:

A 40% suspension in sesame oil 40 g/ad 100 ml) was prepared and administered once by gavage at dose levels to beagle dogs as shown in tabulation below.

Each dosage was tested with one male and one female animal. The animals were deprived of food for 16 hours before and 5 - 6 hours after the treatment.

Results:

Dose mg/kg	Concentration %	Mortalities / Number of dogs
1000	40	0 / 2
1500	40	0 / 2

The findings recorded for the individual animals are indicated in the following survey:

1000 mg/kg: This dose was tolerated by both dogs without any reactions. Behaviour and body weight increments were normal during the follow-up period.

1500 mg/kg: 24 hours after the treatment the female animal showed superactivity and squatting but was free from clinical symptoms after 48 hours. 195 minutes after the treatment the male animal showed abdominal position and emesis but was free from clinical symptoms 24 hours after dosing.

Behaviour and body weight increments of both animals are normal during the follow-up period.

There were no deaths.

Conclusions:

Acute oral LD_{50} (one male and one female dog: More than 1500 mg/kg).

Core Classification:

Supplementary

003682

VI-6

TOXICOLOGY BRANCH
DATA REVIEW

Study Type: Acute intraperitoneal toxicity, male rat

Accession Number: 071787 (A:6)

MRID Number:

Sponsor: American Hoechst Corporation

Contracting Lab: Hoechst Aktiengesellschaft, report no. 421/79

Date: July 27, 1979

Test Material: ethyl-2-(4-(6-chlorobenzoxazolyloxy)-phenoxy) -propanoate, 97%, Fenoxaprop-ethyl, technical (Hoe 33171 O H AT203)

Protocol:

A 12.5% suspension in sesame oil (12.5 g/ad 100 ml) was prepared for the acute treatment and administered once by intraperitioneal injection at various dose levels to male rats as shown in tabulation below.

Results

"The following mortality rate was registered in the various dosage groups after termination of the 14-day follow-up period:

Dose mg/kg	Concentration %	Mortalities / Number of animals
500	12.5	2 / 10
800	12.5	8 / 10
1 250	12.5	7 / 10
2 000	12.5	7 / 10
3 150	12.5	8 / 10
5 000	12.5	10 / 10

Lethally intoxicated animals died between day 1 and 6 after the treatment The following clinical symptoms were recorded: squatting, horripilation, abdominal position and passiveness."

"Marked decrease in weight was recorded 7 days after the treatment in some of the animals dosed with 1250, 2000 and 3150 mg/kg. At the end of the follow-up period the body weight of all animals was definitely above the initial value."

The autopsy of the animals that had died produced the following findings: extreme filling of the stomach with mashy feed and autolysis.

The animals killed after termination of the experiment showed deposits of substance in the abdominal cavity and on the organs. Punctiform white spots were seen on the liver, kidneys, intestine and diaphragm. In addition, liver and suprarenal glands were partly light brown discoloured and the liver showed slight marking."

Conclusions

Intraperitoneal LD_{50} (male rat): 739(253-1159)mg/kg bw.

003682

TOXICOLOGY BRANCH
DATA REVIEW

Study Type: Acute intraperitoneal toxicity, female rat

Accession Number: 071787 (A:7)

MRID Number:

Sponsor: American Hoechst Corporation

Contracting Lab: Hoechst Aktiengesellschaft, report no. 422/79

Date: July 27, 1979

Test Material: ethyl-2-(4-(6-chlorobenzoxazolyloxy)-phenoxy) -propanoate, 97%, Fenoxaprop-ethyl, technical (Hoe 33171 O H AT203)

Protocol:

A 12.5% suspension in sesame oil (12.5 g/ad 100 ml) was prepared for the acute treatment and administered once by intraperitioneal injection at various dose levels to female rats as shown in tabulation below.

Results

"At the end of the 14-day follow-up period the following mortality rate was recorded in the varicus dosage groups:

Dose mg/kg	Concentration %	Mortalities / Number of animals
315	12.5	0 / 10
500	12.5	0 / 10
800	12.5	6 / 10
1 250	12.5	9 / 10
2 000	12.5	10 / 10
3 150	12.5	9 / 10

Lethally intoxicated animals died between day 1 and 3 after the treatment showing the following clinical symptoms: squatting, horripilation, abdominal position and passivenes.

Slight to marked decrease in weight was recorded 7 days after the treatment in some of the animals dosed with 315, 500, 800, 1250 and 3150 mg/kg body weight. At the end of the follow-up period the body weight increments of all experimental animals were normal again."

"The macroscopic post-mortem examination of the animals that had died revealed extreme filling of the stomach with mashy feed and autolysis.

The dissection of the animals that were killed revealed deposites of substance in the abdominal cavity and on the organs. Punctiform white spots or a film-like, thin-layered coat were seen on liver, spleen and kidneys. In addition, the liver was partly light-brown discoloured and showed slight marking."

Conclusions

Intraperitaoneal LD_{50} (female rat): 864(691-1079)mg/kg bw (p=0.05)

TOXICOLOGY BRANCH
DATA REVIEW

Study Type: Acute inhalation, rat (4-hour)

Accession Number: 071787 (A:8)

MRID Number:

Sponsor: American Hoechst Corporation

Contracting Lab: Hoechst Aktiengesellschaft, report no. 352/82

Date: June 8, 1982

Test Material: ethyl-2-(4-(6-chlorobenzoxazolyloxy)-phenoxy) -propanoate, 97%, Fenoxaprop-ethyl, technical (Hoe 33171 O H AT204)

Protocol:

Phenoxaprop-ethyl, technical, "Hoe 33171 O H AT204 was in the form of a lightbrown crystalline powder and was used as a 5% dilution in ethanol/ polyglycol (1:1) to determine the LC_{50}. Rats were exposed to known aerosol concentrations."

Results:

Invalid.

Conclusions:

This study should have been made using powdered technical material, not the solution. See latest guidelines.

Core classification:

Invalid.

TOXICOLOGY BRANCH
DATA REVIEW

Study Type: Acute dermal toxicity, female rat

Accession Number: 071787 (A:9)

MRID Number:

Sponsor: American Hoechst Corporation

Contracting Lab: Hoechst Aktiengesellschaft, report no. 578/79

Date: October 2, 1979

Test Material: ethyl-2-(4-(6-chlorobenzoxazolyloxy)-phenoxy) -propanoate, 97%, Fenoxaprop-ethyl, technical (Hoe 33171 O H AT203)

Protocol:

"A 40% suspension was prepared in sesame oil (40 g/ad 100 ml) and applied once in a concentration of 2000 mg/kg body weight to the shaven dorsal skin of 6 female Wistar rats."

"After the treatment the mechanically shaven and intact dorsal skin (area of exposure appr. 30 cm^2) was covered with a strip of aluminium foil (6 x 8 cm) and secured in position around the trunk of the animal by an elastic plaster bandage (Elastoplast®, 8 cm in width). The dressing was removed after the 24-hour exposure and the treated site was washed with tepid water." Animals were observed 14 days after dosing.

Results

"There were no deaths after the application of 2000 mg/kg body weight. Passivity was observed in all experimental animals until 24 hours after the treatment. Subsequently, behaviour and body weight gains were normal during the follow-up period.

The dissection of the animals killed after termination of the experiment produced no abnormal macroscopic findings."

Conclusions:

Acute dermal toxicity LD_{50} (female rat): more than 2000 mg/kg bw.

Acute dermal toxicity category: III

Core Classification:

Minimum for category III

003682

TOXICOLOGY BRANCH
DATA REVIEW

Study Type: Acute dermal toxicity, rabbit

Accession Number: 071787 (A:10)

MRID Number:

Sponsor: American Hoechst Corporation

Contracting Lab: Hoechst Aktiengesellschaft, report no. 407/79

Date: July 11, 1979

Test Material: ethyl-2-(4-(6-chlorobenzoxazolyloxy)-phenoxy) -propanoate, 97%, Fenoxaprop-ethyl, technical (Hoe 33171 O H AT203)

Protocol:

1000 mg/kg body weight of the substance was applied once to the shaven nape skin of 6 rabbits

"The experiment was performed in such a way that the animals could not lick off the areas of exposure (plastic sleeve around the neck of the animal), thus preventing an oral intake of the test substance. Five hours after the treatment the skin of the rabbits was washed with cold tap water using a sponge-cloth. During the experiment the animals were housed singly in cages."

Results:

No animals died. 24 hours after the treatment the nape skin of all animals was markedly reddened and an average decrease in weight by 103 g (40 - 134 g) was recorded. 48 hours after the application the reddening persisted and the nape skin was also hardened. Three animals showed further decrease in weight while a marked increase was already recorded for the remaining animals. Seven days after the treatment the skin lesions of the rabbits had receded again. All animals showed no abnormalities during the subsequent 7-day follow-up period. No mortalities occurred. The autopsy of the animals killed after termination of the experiment produced no pathological macroscopic findings.

Conclusions:

Acute dermal LD_{50} (rabbit): more than 1000 mg/kg bw.

Acute dermal toxicity category: II

Core Classification:

Minimum for category II labeling. Inadequate for category III or IV.

TOXICOLOGY BRANCH
. DATA REVIEW

Study Type: Primary eye and skin irritation in rabbit

Accession Number: 0781787 (A:11)

MRID Number:

Sponsor: American Hoechst Corporation

Contracting Lab: Hoechst Aktiengesellschaft, report no. 405/79

Date: 7-11-79

Test Material: ethyl-2-(4-(6-chlorobenzoxazolyloxy)-phenoxy)-propanoate, 97%, Fenoxaprop-ethyl,technical (Hoe 33171 O H AT203)

1. Primary dermal irritation

Protocol:

"An area of at least 6 x 8 cm of the flank skin of 6 rabbits each was clipped free of hair. One half of the shorn area was additionally scarified. 500 mg Hoe 33171 O H AT203 (premixed with 0.3 ml polyethylene glycol 400 each) was applied to gauze patches measuring 2.5 x 2.5 cm each. These patches were secured in position by adhesive tapes on the areas prepared for exposure and covered with an indifferent, impervious PVC-foil (6-8 cm in width). Subsequently, the trunk of the animal was bandaged using an elastic permanent dressing (Elastoplast®). The period of exposure was 24 hours. The first evaluation of the irritant effect took place immediately after removal of the dressing and subsequently 48 and 72 hours after the application. The experiment was performed according to EPA Regulations (Federal Register 43, No. 153, 22.8. 1978, § 163.81-5, p 37350). Based on the findings, the irritation index was established according to the classification described in the Federal Register 38, No. 187, 27.9.1973, p 27019, § 1500.41"

Results:

"An irritation index of 1.8 was established after the application of the premixed active substance. All experimental animals showed slight to moderate erythema and edema. The treated skin of 3 rabbits was in addition slightly squamous."

Conclusions:

Primary dermal irritation category: IV

Core Classification:

Guideline

2. Primary eye irritation

Protocol:

Single doses each of 100 mg Hoe 33171 O H AT203 (premixed 0.08 mg each polyethlene glcycol 400) were applied to the conjunctival sac of the left eye of 9 rabbits.

Results:

Invalid.
The 1978 guidelines as well as the current guidelines specify that solids be tested as the dry powder.

Conclusions:

Invalid

Core Classification:

Invalid

003682

TOXICOLOGY BRANCH DATA REVIEW

Study Type: 32-day feeding, rat (range-finding)

Accession Number: 071788 (A17)

MRID Number:

Sponsor: American Hoechst Corporation

Contracting Lab: Hoechst Aktiengesellschaft, report no. 154/80

Date: 6-9-80

Test Material: ethyl-2-(4-(6-chlorobenzoxazolyloxy)-phenoxy) -propanoate, 97%, Fenoxaprop-ethyl (Hoe 33171 OH AT203)

Protocol:

Ten male and 10 female rats per group were administered 0, 80, 315, 1250, or 5000 ppm in feed.

Results

"Because of poor general condition, refusal of feed and decrease in weight, the animals in the 5000 ppm dosage-group were killed on days 7 and 8 of the study.

Behavior and general stage of health were only impaired at concentrations of 1250 and 5000 ppm. Furthermore, significant retardation or decrease of body weight gains were observed in these dosage groups, accompanied by reduced feed and increased water consumption.

The haematological parameters were largely uninfluenced by the administration of the test substance." Decreases in lymphocytes in males were seen at all dosage levels and may have been dose related, but were not statistically significant below the 1250 ppm level.

"The comparison of the clinical chemistry parameters produced the following values of statistical significance:

Sex	Parameter	Dose (ppm)	Deviations from control
♂	cholesterol	80, 315, 1250	Decrease
	total lipids	315, 1250	Decrease
	inorg. phosph.	315, 1250	Decrease
	urea - N	1250	Increase
	AP	1250	Increase

Sex	Parameter	Dose (ppm)	Deviations from control
♀	cholesterol	80, 315, 1250	Decrease
	total lipids	315, 1250	Decrease
	uric acid	315, 1250	Decrease
	potassium	1250	Decrease
	inorg. phosph.	1250	Decrease
	AP	1250	Increase

"Compared to the values of the controls a statistical change in the following relative organ weights was seen in the post-mortem examinations.

Sex	Organ	Dose (ppm)	Change compared to controls
♂	Liver	315, 1250	Increase
	Kidneys	80, 1250	Increase
	Testes	1250	Increase
	Brain	1250	Increase
♀	Liver	315, 1250	Increase
	Kidneys	80, 315, 1250	Increase
	Adrenals	1250	Decrease
	Brain	1250	Increase

"The urinalyses were not indicative of substance-induced changes."

"Significant increases in the absolute and relative weights of the liver were seen in the 315 and 1250 ppm groups. These findings correlated with the histopathological examinations where eosinophilic, fine-granulated and partly distinctly enlarged hepatocytes were found. Necroses of the liver were observed in the animals of the 5000 ppm group that were killed prematurely. No further pathological changes in the organs were found."

Conclusions:

A 32-day NOEL was not found. Extensive toxic effects were observed in the 1250 and 5000 ppm groups. Changes in livers and kidneys and altered lipid metabolism were observed at lower dosage levels. Since a NOEL was not found, this study was inadequate for its intended purpose, range-finding.

Core Classification: "Minimum"

01-13

TOXICOLOGY BRANCH DATA REVIEW

Study Type: 3-months feeding, rat

Accession Number: 071789 (A18)

MRID Number:

Sponsor: American Hoechst Corporation

Contracting Lab: Hoechst Aktiengesellschaft, report no. 695/81

Date: 12-4-81

Test Material: ethyl-2-(4-(6-chlorobenzoxazolyloxy)-phenoxy)-propanoate, 96%, Fenoxaprop-ethyl (Hoe 33171 OH AT204)

Protocol:

"Fenoxaprop-ethyl was administered to rats (30 males and 30 females per group) in the daily feed for 3 months at concentrations of 0, 20, 80 and 320 mg/kg diet/day (ppm). 10 male and 10 female animals per group were kept for a 4-week recovery period."

"Behavior and general state of health were assessed daily. Once a month the rats were examined for neurological disorders, cloudiness of ocular media, disorders in dental growth and changes of the oral mucosa."

"The body weight was recorded once a week."

"The food consumption was checked together with the body weight."

The water consumption was determined at 14-day intervals. The indicated values represent the 16-hour consumption.

"Haematological examinations were made prior to the beginning of the study at week 6 and 13 of the study."

The blood samples of 10 ♂ / 10 ♀ non-fasted rats were withdrawn from the retroorbital venus plexus.

The following parameters were examined:

Haemoglobin
Erythrocytes
Haematocrit
Reticulocytes *
Heinz bodes *

Leucocytes
Thrombocytes
Coagulation time
Differential blood count

*Examined only at the end of the study."

"Further parameters were calculated by data processing:

MCV = median cell volume
MCH = median corpuscular haemoglobin content
MCHC = median corpuscular haemoglobin concentration.

The Met-haemoglobin (1.11.) of 10 ♂ / 10 ♀ rats in test group 4 was determined at week 6 and 13 of the study."

"Serum analyses were made in 10 non-fasted rats per group and sex prior to the beginning of the study. The following parameters were determined:

Glucose
Urea-N
SGOT
SGPT
alkaline phosphatase"

"The following parameters were determined in 10 ♂/ 10 ♀ non-fasting rats at week 6 of the study (intermediate values):

Sodium
Potassium
Inorg. phosphorus
Uric acid
Total bilirubin
Glucose
Creatinine
Urea-N
Calcium
Chloride

"The following examination was made during week 6 of the study in 10 ♂/10 ♀ rats.

SGOT
SGPT
Alk. phosphatase
Total protein
Total lipids
Cholesterol
LDS
Bilirubin direct
Electrophoresis

(Protein, α_1 -, α_2 -, α_3 -Globuline
β_1 -, δ_1 -Globuline

"At week 13 of the study (final value) the above mentioned parameters were determined in the serum of 20 ♂ / 20 ♀ animals obtained from exsanguination."

"The remaining animals were examined accordingly after a 4-week follow-up period."

"After the animals were deprived of food and drinking water, their urine was collected overnight in diuresis cages. The urine of 10♂/10♀ animals per group was analyzed prior to the beginning of the study (initial value), at week 6 (intermediate value) and at week 13 of the study (final value)."

"The following parameters were determined:

Appearance
Color
Protein
Glucose
Haemoglobin
Bilirubin
pH-value
Sediment
Specific weight
Ketone bodies
Urobilinogen

"3 months after the beginning of the study 20♂ / 20♀ rats were killed 24 hours, the remaining animals 4 weeks after withdrawal of the test substance under Pentobarbital-sodium-anaesthesia (50 mg/kg i.p.) and exsanguinated after severing of the Vena cava cranialis."

"Integument, orifices, eyes and viscera were gross-examined on dissection. Findings deviating from normal were registered in the autopsy records."

"The following organs were removed, weighed and conserved in fixative:

Heart
Lungs
Liver
Kidneys
Spleen
Brain
Both testes/ovaries
Adrenals
Pituitary
Seminal vesicle
Thyroid"

"The remaining organs or parts of these organs were conserved without indication of weight:

Thymus
Salivary glands (Parotis and Mandibularis)
Trachea, oesophagus
Stomach (fundus and prepyloric region)
Intestine (duodenum, jejunum, ileum, coecum, colon, rectum)
Urinary bladder, prostate, epididymes, uterus
Pancreas
Abdominal aorta
Diaphragm
Eyes with optic nerves
Skeletal muscle
Marrow of the femur
Lumbar vertebra
Lymph nodes (Hilus and Iliacus)
Skin with mammary gland
Tumors (if any)
Spinal marrow with sciatic nerve.

"The organs or parts of organs removed on dissection were conserved in fixative and submitted to histological examination.

Results:

"All animals survived to the scheduled end of the study."

"The behavior of the rats was not influenced by the test-substance throughout the study.

Neurological disorders, cloudiness of ocular media, disorders in dental growth or changes of the oral mucosa, attributable to the oral administrtion of Hoe 33171 were not observed."

"The body weight gains were normal and not influenced by the test-substance."

"The food consumption showed no differences between treated and untreated animals."

"The relative water consumption of the male rats and the female animals in the 320 ppm group was slightly lower throughout the study than that of the untreated animals."

"The haematological examinations revealed no harmful influences of the test substance."

"The results of clinical chemistry indicate a substance-induced change in the lipid status manifesting itself in the serum cholesterol of the males (80 and 320 ppm group) and in the cholesterol (320 ppm group) and total lipid content (20, 80, 320 ppm) in the serum of the females."

A significant increase in alkaline phosphatase-values in the male rats (320 ppm-group) was observed.

"The urinalyses were not indicative of any adverse effect of the test-substance." Increased turbidity was seen in urine of high dose level.

"During the study the following relative organ weights were increased as compared to the values of the controls." Changes marked with an asterisk were statistically significant.

Organ	Sex	Percent increases of dosage groups 20	80	320
Liver	male	-0.15	0.10	15.5*
Kidney	male	0.69	1.73	10.7*
Thyroids	male	10.6	14.9	21.3*
Kidney	female	1.99	4.59	6.29*
Adrenals	female	10.1	15.2*	19.5*
Ovaries	female	15.0*	11.5	13.0

Changes appear to be dose related at all dosage levels in male kidneys and thyroids and in female kidneys and adrenals.

"No substance-induced macroscopic organ changes were seen. The changes - particularly in the kidneys - indicated under individual findings are considered spontaneous alterations and appear in treated and untreated rats."

"From the groups dosed with 20 and 80 ppm only the liver and the organs with macroscopic findings were examined histologically."

"The histological examinations revealed that the administration of the high dosage of 320 ppm in the daily feed produced moderate enlargement of the centrobular hepatocytes in the male rats. The cytoplasm of these cells was eosinophilic and finely granulated."

"The administration of 20 and 80 ppm produced no lesions in the livers of male and female rats."

"After the 4-week follow-up period all organ weights were inconspicious except for the significantly decreased weights of the liver of the female rats of the 80 ppm group and the males of the 20 ppm dosage group."

Conclnsions:

Although the above apparent organ weight increases at the 20 ppm level are not confirmed statistically they indicate cause for concern. 20 ppm has been accepted as the NOEL for use to support the EUP, but a clearer NOEL is neeed from the 2-year study to support registration. The issues raised by the apparent effects should be addressed in the report on the two year study.

NOEL: 20 ppm

LEL: 80 ppm (relative organ weight changes)

Core Classification:

"Minimum"

003682

CL-14

TOXICOLOGY BRANCH DATA REVIEW

Study Type: 32-day feeding toxicology mice (Range-finding)

Accession Number: 071790 (A19)

MRID Number:

Sponsor: American Hoechst Corporation

Contracting Lab: Hoechst Aktiengesellschaft, report no. 336/80

Date: 6-10-80

Test Material: ethyl-2-(4-(6-chlorobenzoxazolyloxy)-phenoxy) -propanoate, 97%, Fenoxaprop-ethyl (Hoe 33171 OH AT203)

Protocol:

"The following concentrations were tested in 5 groups, each consisting of 10 male and 10 female animals, 0, 80, 315, 1250 and 5000 ppm."

Results:

"The animals in the 5000 ppm dosage group were killed on days 8 and 9 of the study because of poor general condition, refusal of feed and decrease in weights.

Behavior and general state of health were unimpaired throughout the study after the administration of the test substance in concentrations of 80, 315 and 1250 ppm."

"At termination of treatment the haematological examinations showed only a statistically significant reduction of haemoglobin in male and female animals of the 1250 ppm dose group. However, there was no further evidence for any type of anaemia, because all other haematological and clinico-chemical parameters as well as the histomorphological investigations of spleen and bone marrow did not show any deviation from the controls."

"At dose levels of 80 and 315 ppm both the total lipids and cholesterol concentrations in the serum were significantly increased, whereas at 1250 ppm no deviations from control values could be detected."

"The increase in alkaline phosphatase (AP) and serum glutamic pyruvic transaminase (SGPT) was considered to be substance-related and toxicologically relevant. These dose-dependent findings were seen at 315 ppm and higher concentrations."

Smaller increases in SGOT were not statistically significant.

"Initially a statistically significant retardation in body weight development was observed in the 1250 ppm-group. The body weights in this test group returned to normal and those in the other groups were not affected by treatment."

"Significant increases in the absolute and relative weights of the liver were seen in all treated groups. The female animals dosed with 315 and 1250 ppm showed increased weights of kidneys. These findings correlated with the histopathological changes in liver and kidneys. Eosinophilic, fine-granulated and partly distinctly enlarged hepatocytes were seen, intensifying with increasing concentrations. Additional necroses of the liver were observed in the animals of the 5000 ppm group that were killed prematurely. Furthermore, tubular lesions in the kidneys were detected only in the female animals with 315 ppm and higher concentrations."

"The increase in alkaline phosphatase described above is a further indication of a liver toxicity."

Conclusions:

Toxicity was detected at all dosage levels. A NOEL was not found.

Core Classification:

This study is supplemental to the 30-day range-finding study which followed it, no. 356/81.

TOXICOLOGY BRANCH DATA REVIEW

Study Type: 30-day feeding, mice (second range-finding study).

Accession Number: 071791 (A20)

MRID Number:

Sponsor: American Hoechst Corporation

Contracting Lab: Hoechst Akliengesellschaft, report no. 356/81

Date: 6-24-81

Test Material: ethyl-2-(4-(6-chlorobenzoxazolyloxy)-phenoxy) -propanoate, 96%, Fenoxaprop-ethyl (Hoe 33171 OH AT204)

Protocol:

"The following concentrations were tested in 5 groups, each consisting of 10 male and 10 female animals, 0, 5, 10, 20 and 80 ppm."

Results:

"No animals died during treatment."

"Behavior and general state of health were unimpaired throughout the study after the administration of all concentrations of the test substance in the feed."

"The haematological parameters remained uninfluenced by the treatment with the test substance."

Increases in cholesterol were seen in females of the 20 and 80 ppm groups.

Apparently dose-related increases in total lipids and cholinesterol were seen in males of all groups. However, the changes were too small to be statistically significant. Also apparently dose-related decreases in SGOT and SGPT were seen in females of the 20 and 80 ppm groups. These changes were also not statistically significant.

"Protein was seen in a few animals dosed with 20 and 80 mg/kg. It was evident from the previous study, 336/80, that higher concentrations, 315 and 1250 ppm, produced tubular lesions. The content of protein in the urine in this study is also indicative of a renal lesion."

No histopathology was reported from this second range-finding study.

"The body weight gains of the animals were normal in all dosage groups throughout the study."

"Significant increases in the liver weights (15% and 19%, respectively) were observed in the females dosed with 20 and 80 ppm and in the males of the 80 ppm group (22%). These findings correlated with the histomorphological examinations where dose-dependent changes in the form of enlarged hepatic epithelia with relatively large nuclei and dense eosinophilic cytoplasm were seen in the centrolobular sections of the liver. No indication of liver cell necroses was seen at any of the other concentrations tested." Note that the previous study in mice, no. 336/80, found necrosis of livers at highest dosage level, 5000 ppm.

"There was an obvious sex specific reaction: Liver changes in the males were more pronounced than in the females."

30-day NOEL: 10 ppm
30-day LEL: 20 ppm (increased liver weight gains)

Conclusions:

Fenoxaprop-ethyl can cause liver and kidney damage.

Acceptable as a range-finding study.

Core Classification: "Minimum"

LL-16

TOXICOLOGY BRANCH DATA REVIEW

Study Type: 30-day feeding, dog (range-finding)

Accession Number: 071792 (A21)

MRID Number:

Sponsor: American Hoechst Corporation

Contracting Lab: Hoechst Aktiengesellschaft, report no. 165/80

Date: 3-3-80

Test Material: ethyl-2-(4-(6-chlorobenzoxazolyloxy)-phenoxy) -propanoate, 97%, Fenoxaprop-ethyl (Hoe 33171 OH AT203)

Protocol:

Two male and 2 female dogs per group were given 0, 80, 400, or 2000 ppm fenoxaprop in the diet, 7 days per week, 30 days.

Results:

"The dogs in the high-dosage group (2000 ppm) had to be killed in moribund state on day 3 and 5 of the study, respectively. All dogs from the remaining groups lived up to the scheduled end of the study."

"The general state of health in the remaining groups and also in the controls was not impaired. The feed consumption was only affected adversely in one animal in group IV (2000 ppm).

The clinical toxic manifestations of the dogs in the high-dosage group (2000 ppm) were characterized by asynchronism and general weakness."

"No test-induced influence on the general condition was observed in the dogs from 80 or 400 ppm groups."

"Probably due to reduced fluid intake, signs of haemoconcentration were seen in the high-dosage group (increase in erythrocytes, haemoglobin concentration, haematocrit, total number of leucocytes. The findings in the other groups remained unchanged."

"In the 2000 ppm group, the final values of total lipids were about twice the values in all other groups (including control) but still within the range of variation of the initial values."

"In the same group (2000 ppm) the SGOT activities in 3 dogs were slightly and the alkaline phosphatase-values in four dogs were markedly increased. Slight increases of β_7 - and γ-globulin-fractions were also seen in the 2000 ppm group."

The 80 and 400 ppm groups revealed no substance-induced changes in clinical chemistry test values.

"No test-induced changes were observed except for increased specific weights in the male dogs of the 2000 ppm group."

"The mean weights of the adrenals in the 400 ppm group were slightly above and the 2000 ppm group distinctly above the mean adrenal weights of controls."

"All animals in the 2000 ppm group showed distinct lobular marking of the liver; in 3 cases the organ was clay-brownish discoloured and the wall of the gall bladder of 3 animals showed subserous haemorrhages. In addition the iliac lymph nodes in all dogs of this group and all visible lymph nodes of the bitch 842 were enlarged."

"There were no substance-induced morphological changes in the 80 ppm-group."

"Microscopy revealed in the 400 ppm group a small focus of siderosis in the general section of the lung, a disputable atrophy of the thymus and hyperplasia of lymph follicles in the thyroid. The male dog No. 833 also shows a slight but bilaterial focal atrophy of a few testicular canaliculi. It could not be excluded that these changes might be substance-induced."

"The dogs in the 2,000 ppm group showed fatty degeneration of the liver, atrophy of splenic corpuscles, acute lymphadenitis, haemorrhages of the adrenal cortex, thymus atrophy and changes of the cerebellum."

Comments:

No effects of treatment were demonstrated in the 80 ppm group after 30 days of treatment in this range-finding test.

Core Classification :

Acceptable as range-finding study

TOXICOLOGY BRANCH DATA REVIEW

Study Type: 3-months feeding toxicity, dog

Accession Number: 071793 (A22)

MRID Number:

Sponsor: American Hoechst Corporation

Contracting Lab: Hoechst Aktiengesellschaft, report no. 674/81

Date: 11-24-81

Test Material: ethyl-2-(4-(6-chlorobenzoxazolyloxy)-phenoxy)-propanoate, 96%, Fenoxaprop-ethyl (Hoe 33171 OH AT204)

Protocol:

Six male and 6 female dogs per group were each administered 0, 16, 80, or 400 ppm fenoxaprop in feed. Four per sex per group were killed at end of dosing. The others were killed 4 weeks after end of treatment.

There were daily assessments of general condition, feed consumption and behavior and weekly weighings.

Neurological conditions were assessed by "testing, flexor, patellar, anal, cutaneous, corneal, pupillary and blink reflexes, and of placing (visual and tactile), and righting reflexes before the first dosing, after about 6 weeks, before end of dosing and before end of recovery."

Eyes were examined "with an Okulus hand slit lamp, a Kowa RC2 Fundus Camera and a Zeiss slit-lamp microscope before the first dosing, after about 6 weeks and before end of dosing."

"The examination included the following parts of the eye: cornea, anterior chamber, iris/pupil, lens, vitreous body and fundus.

The examinations were performed in a darkened room with pupils dilated."

"Hearing was monitored by simple noise tests before start of study, after about 6 weeks, before end of dosing and before end of recovery."

"Blood samples were taken and hematological values determined before start of study (initial values), after about 4 and

8 weeks (intermediate values), before end of dosing (final values) and about 4 weeks after end of dosing in the recovery animals (i.e. by the end of the recovery period).

The examinations covered:

Hemoglobin	Heinz bodies
Erythrocytes	Differential blood count
Leukocytes	
Hematocrit	Thrombocytes
Reticulocytes	Coagulation time"

"The following parameters were determined in all animals of each group:

Sodium	Cholesterol
Potassium	Total glycerol
Inorganic phosphorus	(triglycerides)
Uric acid	Total lipids
Total bilirubin	Total proteins
Direct bilirubin	Electrophoresis
(except initial values)	
Creatinine	Methemoglobin (only final values)
Serum glucose	SGOT
Urea nitrogen	SGPT
Calcium	Alkaline phosphatase
Serum iron	LDH"

"The following parameters were determined in the urine collected from each animal.

Appearance
Color
pH
Protein
Glucose
Hemoglobin
Bilirubin
Ketone bodies
Specific weight
Sediment
Urobilinogen"

The sulfobromophthalein sodium tests (BSF) were used to test liver function.

Phenolsulfonphthalein tests (PSP) were performed to assess liver function before start of study, at end of dosing and at end of recovery.

"The following organs were weighed:

Heart	Spleen	Ovaries/Testes	
Lungs	Brain	Adrenals	
Liver	Pituitary	Thyroid	
Kidneys	Pancreas	Thymus	Not covered by electronic data processing."
		Prostate	

No values for thymus or prostate were found in report.

"The following organs or organ parts were removed and fixed in formaldehyde solution and/or Carnoy's fluid for microscopic examination:

Heart	Stomach (fundus and prepyloric region)
Lungs	Duodenum
Liver	Jejunum
Kidneys	Ileum
Spleen	Cecum
Adrenals	Colon
Thyroid	Rectum
Pancreas	Gall bladder
Thymus	Tonsils
Pituitary	Salivary glands (parotid and submandibular)
Cerebral Cortex	
Brain stem	Lymph nodes (cervical and iliac)
Cerebellar cortex and medulla	Esophagus
Medulla oblongata	Trachea
Eyes with optic nerves	Aorta (thoracic)
Urinary bladder	Diaphragm
Testes/Ovaries	Skeletal muscle (psoas)

Epididymides/Uterus Skin with mammary gland

Prostate

Midsternal bone marrow (fixed in Schaffer's solution)"

Results:

"All dogs survived up to the scheduled end of study."

"The dogs always consumed their feed rations completely; the feed consumption was thus not affected by any circumstances of the study."

"The body weight development was not affected by the study. The curves of the treated groups corresponded to those of the control group."

"The behavior of the dogs was unremarkable."

"The testing of reflex excitability and postural reactions revealed no changes from the initial findings."

Study-induced changes were not observed in eyes or hearing.

Hematological and clinical chemistry values were within physiological limits. Statistical differences from controls are mostly found in the highest dosage group (400 ppm) and all are of doubtful toxicological value.

"The methemoglobin tests yielded negative findings."

Liver function tests revealed no pathological retention.

Renal function tests revealed no reduction in tubular excretion.

"Compound-induced macroscopic organ changes were not observed."

The significant increase, as compared to controls, of pituitary weights is unexplained. The histological examination of pituitaries revealed no abnormal findings. No weights of thymus were reported.

"On the basis of the results of histological examinations, the dogs treated with 16 mg per kg feed remained free of morphologically detectable organ changes whereas 80 and 400 mg per kg feed obviously induced chronic interstitial pyelonephritis or at least promoted its development."

Conclusions:

NOEL 16 ppm
LEL 80 ppm (inflamatory changes in kidneys)

Core Classification: Minimum

TOXICOLOGY BRANCH
DATA REVIEW

Study Type: Teratology and enbryotoxicity study, rat

Accession Number: 071794 (A:23)

MRID Number :

Sponsor: American Hoechst Corporation

Contracting Lab: Hoechst Aktiengesellschaft, report no. 613/82

Date: October 4, 1982

Test Material: ethyl-2-(4-(6-chlorobenzoxazolyloxy)-phenoxy) -propanoate, 93%, Fenoxaprop-ethyl, technical (Hoe 33171 O H AT204)

Protocol

"The technical constituent, Hoe 33171 O H AT204, dissolved in sesame oil was administered orally by tube to groups of 20 female Wistar rats from the 7th - 16th day of pregnancy once a day in doses of 10, 32 and 100 mg/kg body weight. At the same time, control animals received the vehicle with no compound additive. On the 21st day of pregnancy, the dams were killed and delivered" by caesarean section.

"After iron staining the uterus with ammonium sulphide, all implantation sites in the uterus were counted."

"Subsequently, the foetuses were examined for external appearance and externally visible abnormalities.

About half of the foetuses from each litter as well as the dead foetuses, also known as skeletal foetuses, were fixed in alcohol, dissected under a magnifying glass, eviscerated and then cleared in potassium hydroxide solution. The skeletons were stained with alizarin red S and examined under a stereomicroscope for level of developement and any abnormalities."

"The other foetuses, also known as cross-section foetuses, were fixed in Bouin's solution and examined in transverse body cross-sections under a steromicroscope for organ abnormalities.

The foetuses were selected for the two examinations alternately according to their position in the uterus.

The dams were dissected after caesarean section and their organs submitted to macroscopic examination. Heart, liver, kidneys and spleen were weighed."

Results:

"All of the dams survived the end of the study. No distrubance in the behaviour or general state of health of the dams in the 10 mg/kg and 32 mg/kg groups could be discovered."

"The administration of 10 and 32 mg Hoe 33171 per kg body weight did not lead to any disturbance of general condition, feed consumption or body weight increase in the dams, nor did it adversely affect the development of the conceptuses in the uterus."

"On administration of 100 mg/kg, the animals showed initial signs of intolerance in the form of a slight reduction in feed consumption and reduced weight increase; piloerection was observed in 6 of the 40 dams. In addition, caesarean section showed that 4 of the 40 dams had only implantation sites in the uterus."

"The foetuses delivered alive in the 100 mg/kg group exhibited slightly impaired growth and reduced placental weights."

"The skeletons of the live foetuses in the 10 mg/kg and 32 mg/kg groups were at the same stage of development as those of the control foetuses, appropriate to the 21st day of pregnancy." In contrast, in addition to "reduced body weights, ossification of the skeleton in the foetuses from the 100 mg/kg group (group 4) was slightly retarded. This was particularly evident from the cranium and from the deficient ossification of the sternebrae and the 5th metacarpal."

All abnormalities which were not spontaneous were attributable to fetotoxicity.

Conclusions:

Teratogenicity: $\geq$ 100 (HDT)

Maternal toxicity NOEL: 32 mg/kg
LEL : 100

Fetotoxicity NOEL: 32
LEL: 100

Core Classification

Minimum

003682

TOXICOLOGY BRANCH
DATA REVIEW

Study Type: Metabolism: kinetics and residue determinations after oral and intravenous dosing in rats.

Accession Number: 071794 (A:24)

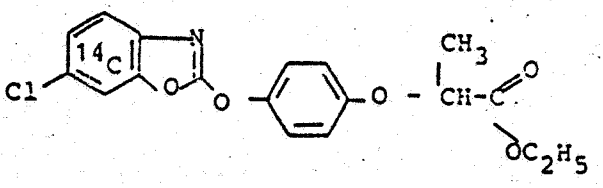
MRID Number:

Sponsor: American Hoechst Corporation

Contracting Lab: Hoechst Aktiengesellschaft, report no. 01-L42-0364-82

Date: 4-22-82

Test Material: ethyl-2-(4-(6-chlorobenzoxazolyloxy)-phenoxy)-propanoate, Fenoxaprop-ethyl-C^{14} (HOE 33171 OHAT 102) diluted with unlabelled Hoe 33171 (code no. HOE 33171 OHAT 302) in the ratio 1:9 (specific radioactivity of the diluted preparation: 2.6 mCi/g



Protocol:

"Studies were to be conducted on rats to learn about the behavior of the compound in the rat in respect of enteral absorption and elimination and also about the residual concentrations in the rat organs and tissues at the trials' end."

Phenoxaprop-ethyl was administered to 5 male and 5 female rats by the intravenous and oral routes at a dose of about 2 mg/kg. After treatment, the rats were maintained in metabolism cages. Samples of blood, urine, feces, and expired air were processed and measured for radioactivity. Radioactivity was also determined in organs of rats dosed orally.

"For intravenous injection the preparation was dissolved in polyethlene glycol 400. The solution when ready for injection had a concentration of about 0.5 mg/ml. For oral application Hoe 33171-^{14}C was given an admixture of 2% starch paste and ground wet for 30 min. with steel balls in an agate mortar on a planetery mill ("Pulverisette 5," A. Freitsch OHG, Idar Oberstein, W., Germany) and then with stirring diluted with starch paste to final concentration of about 0.5 mg/ml."

"The blood samples were taken from the retrobulbar venous plexus. During the trial the animals were kept in individual metabolism cages, from which feces and urine can be collected separately.

In order to record the release of radioactivity with the respiratory air, one female and one male were kept in sealed metabolism cages, through which a stream of air was drawn. The radioactivity in the expired air was meaured continuously with the help of a gas-flow meter (Exhalameter type FHT 50 B, Frieseke & Hoepfner company, Erlangen) connected on the outlet side.

To investigate the residues in the organs and tissues, the rats were stunned by a blow on the head and then killed by exsanguination. The post-mortem examination was carried out immediately therafter."

Results:

1. Blood concentrations

"The maximum blood level after oral dosing occurred after eight hours in each sex. The decline in concentration proceeded in two phases with biological half-lives of about 15 hours (males) and six hours (females) for the first phase and about 75 hours in both sexes for the second phase." Resulting blood concentrations are shown graphically in figures 2 and 3.

2. Elimination in urine and feces

The elimination is presented graphically in figs. 4 and 5.

"Regardless of the application route, more radioactivity was eliminated in the urine than in the feces. The proportion eliminated renally was greater for the females than for the males.

Table 12: Synopsis of the radioactivity eliminated following oral and intravenous application of Hoe 33171-^{14}C

	Per cent of applied dose			
	Oral		Intravenous	
	Males	Females	Males	Females
Urine[1]	54.0 ± 3.4	71.3 ± 8.2	40.9 ± 7.3	62.8 ± 2.5
Feces	44.0 ± 2.5	25.3 ± 2.5	33.1 ± 5.6	23.5 ± 2.0
Total	98.0 ± 3.0	96.6 ± 7.5	74.0 ± 11.8	86.3 ± 3.6

[1] Including rinse liquid from cages, which contains the urine that dried on the cage walls"

3. Elimination in the respiratory air

"For one male and one female rat the radioactivity eliminated in the respiratory air was monitored continuously for 24 h following the oral application of 40 mg Hoe 33171-^{14}C per kg bodyweight. No values exceeding the limits of sensitivity of 0.01% of the applied radioactivity were determined."

4. Residual concentrations in organs and tissues 7 days after application

Tables 15 and 16 show the tissue concentrations determined for the male and female rats at the end of the trial.

"In the tissue distribution studies seven days after oral dosing, the kidneys exhibited the highest concentrations of radioactivity followed by retroperitoneal fat and liver. The concentration in these organs was less that in the concentration in the blood."

Conclusions:

Blood concentration levels after either oral or intravenous application peaked after 8 hours.

No radioactivity was detected in respired air.

During 7 days the radioactivity was largely excreted in urine and feces after exposure by either route. Refer to table 12. More was eliminated in urine than in feces. Of the radioactivity not eliminated and therefore remaining in the animals, the highest concentrations were found in the blood followed by that in kidneys, then in retroperitoneal fat and liver. Refer to tables 17 and 18. Total residual radioactivity detected in male rats was 3.31% of ingested doses and in female rats 2.23%.

Excretion curves for the routes of treatment (Figs. 4 and 5), were quite similar both as to shape (reflecting rates of excretion) and area under the curves (indicating quantities excreted). These correlations indicate completeness of absorption from the gastrointestinal tract.

Core Classification:

Minimum

Fenoxaprop-ethyl Toxicology Reviews

Page _____ is not included. The page contains detailed test methods/results submitted by the pesticide registrant.

Pages 52 through 57 are not included. The pages contain detailed methods/results submitted by the pesticide registrant.

003682

LL-20

TOXICOLOGY BRANCH
DATA REVIEW

Study Type: Metabolism: determination of metabolites

Accession Number: 071794 (A:25)

MRID Number:

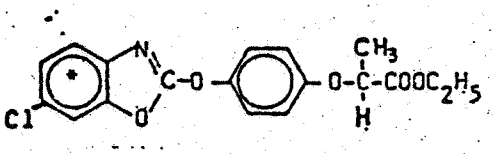
Sponsor: American Hoechst Corporation

Contracting Lab: Hoechst Aktiengesellschaft, Report no. FO.318/82

Date:

Test Material: ethyl-2-(4-(6-chlorobenzoxazolyloxy)-phenoxy)-propanoate, 97%, Fenoxaprop-ethyl (Hoe 33171-^{14}C)

Structural formula:



* Position of labeling with carbon-14

Protocol:

"Carbon-14 ring labeled HOE 33171 was dissolved in salad oil and administered to female SPF-Wistar rats at a single oral dose of 40 mg/kg. The animals were maintained in metabolism cages for the collection of urine and feces. Thin layer chromatography, gas chromatography, mass spectrometry and nuclear magnetic resonance were used to isolate and identify the metabolites. Samples were collected over the following periods: 0-24 hours, 24-48 hours and 48-96 hours."

1. In urine

Considering the loss of small portions of the metabolite in every purification step, Hoechst chemists concluded that mercapturic acid of 6-chlorobenzoxol accounted for more than 50% of the activity excreted in the urine.

"A small amount of 6-chloro-2.3-dihydro-benzoxazol-2-thione, which amounted to 4.3% of the total radioactivity in the urine, was found to be free. But this compound is assumed to be an artificial fragment from its mercapturic acid. This conclusion is in agreement with the high lability of the thioether bond.

Another metabolite was identified to be hydroxylated 6-chloro-2,3-dihydro-benzoxazol-2-one (UD2B) which amounted to 3.1% of the total radioactivity in the urine.

The third degradation product which could be identified was 6-chloro-2,3-dihydro-benzoxazol-2-one.

All the metabolites which were not extractable from the acidified water phase (uC/UA) remained unidentified because of their low amount. In addition, it was not possible to form derivatized compounds thereof, which could be analyzed by use of gas chromatography coupled to mass spectrometry."

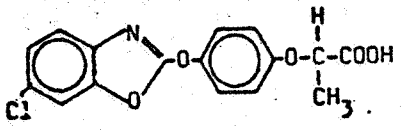
2. In Feces

"66% of the total radioactivity in the feces was extracted and the metabolites therein were characterized. The unchanged parent compound represented 12% of the radioactivity in the feces."

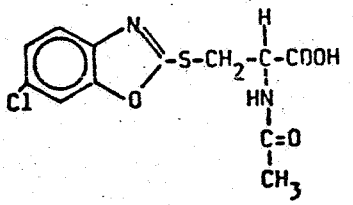
"Two other metabolites were extracted from neutral and acidified water, respectively, the original form of which could not be identified. In both metabolites, 6-chlorobenzoxazol was shown to be a structural element weakly bound to unknown structures. This labile bond was easily cleaved during methylation by use of diazomethane. This phenomenon was not found on methylation of Hoe 053022, the free carboxylic wherein 6-chloro-benzoxazol is bound to a phenyl ring via an oxygen ether bridge. On the other hand the thioether bond of the main metabolite in urine was found to be labile. Therefore these data allow to assume that the main metabolites in feces contain the 6-chloro-benzoxazol bound to a biogenous molecule."

Following are some structural formulas of chemicals referred to above, as presented by Hoechst.

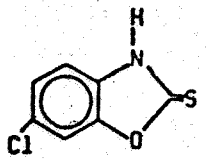
Hoe 53022



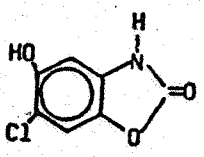
Mercapturic acid 6-chlorobenzoxazol, or
N-acetyl-S-(2-(6-chlorobenzoxazoly)-cysteine



6-chloro-2,3-dihydro-benzoxazol-2-thione

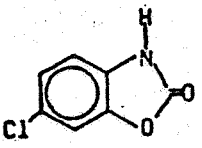


6-chloro-5-hydroxy-2,3-dihydro-benzoxazol-2-one



Position of the hydroxyl group is proposed

6-chlor-2,3-dihydro-benzoxazol-2-one



Conclusions:

12% of radioactivity in the feces was found to be unchanged fenoxaprop-ethyl. Much but not all of the remaining radioactivity in urine and feces was characterized chemically as metabolites as shown above. Over 42% in urine and 34% in feces was not characterized.

Core Classification:

Minimum —

003682

01-21

TOXICOLOGY BRANCH DATA REVIEW

Study Type: Acute oral LD_{50}, male rats

Accession Number: 071795 (CI B:1)

MRID Number:

Sponsor: American Hoechst Corporation

Contracting Lab.: Hoechst Akliengesellschaft, report no. 687/82

Date: November 2, 1982

Test Material: ethyl-2-(4-(6-chlorobenzoxazolyloxy)-phenoxy)-propanoate, Fenoxaprop - ethyl, EC 12.5 (Hoe 33171 OH EC037).

Protocol: Fenoxaprop - ethyl, EC 12.5 as a 25% emulsion in demineralized water (25g/100ml) was administered in varying dose-volumes by stomach tube to male rats. Dose levels and number of animals per group are shown in the table below.

"The following mortality rate was noted in the various dosage groups at the end of the 14 day follow-up period:

Dose mg/kg	Concentration %	Animals died/used
2 000	25	0/10
3 150	25	5/10
4 000	25	7/10
5 000	25	10/10

"The lethally intoxicated animals died between 4 hours and 2 days after treatment. The following symptoms were observed: passivity, balance disorders, crawling or crouching stance, abdominal or lateral position, retracted flanks, hyporeflexia, narrowed palpebral fissure, chromodacryorrhea and noisy breathing. All symptoms had disappeared after 24 hours. No symptoms were shown by the animals in the 2 000 mg/kg group.

"The macroscopic findings showed stomach filled with substance and coloured yellow-white, adrenals dark-brown coloured, pancreas showed diffuse reddening. The behaviour and the body-weight development of the surviving animals were normal during the follow-up period.

"The dissection of the animals killed at the end of the study showed the following in the 3 150 and 4 000 mg/kg dosage group: pancreas with diffuse reddening, adrenals dark-brown coloured. No findings were made in the 2 000 mg/kg dosage group.

"The oral median lethal dose (LD_{50}) for male rats is 3 310 mg/kg; the limits of confidence at p = 0.05 are 2 770 - 3 740 mg/kg body weight."

Conclusions: Acute oral LD_{50} for male rats: 3310 mg/kg (2770-3740 mg/kg body weight confidence limits at p = 0.05). There were no deaths at 2000 mg/kg. Oral toxicity category: III

Core Classification: Minimum

003682

TOXICOLOGY BRANCH DATA REVIEW

Study Type: Acute oral LD_{50}, female rats

Accession Number: 071795 (CI B:2)

MRID Number:

Sponsor: American Hoechst Corporation

Contracting Lab.: Hoechst Akliengesellschaft, report no. 688/82

Date: November 2, 1982

Test Material: ethyl-2-(4-(6-chlorobenzoxazolyloxy)-phenoxy)-propanoate, Fenoxaprop - ethyl , EC 12.5 (Hoe 33171 OH EC037).

Protocol: Fenoxaprop - ethyl , EC 12.5 as a 25% emulsion in demineralized water (25g/100ml) was administered once in varying dose-volumes by stomach tube to female rats. Dose levels and number of animals per group are shown in the table below.

"The following mortality rate was noted in the various dosage groups at the end of the 14 day follow-up period:

Dose mg/kg	Concentration %	Animals died/used
1 600	25	0/10
2 500	25	0/10
2 800	25	3/10
3 150	25	6/10
4 000	25	5/10
5 000	25	10/10

"The lethally intoxicated animals died between 270 minutes and 6 days after treatment. The following symptoms were observed: passivity, stupor, staggering gait, balance disorders, squatting, abdominal or lateral position, retracted flanks, hyporeflexia, piloerection, narrowed palpebral fissure and noisy breathing. All symptoms had disappeared after 72 hours.

"The macroscopic findings showed: stomach filled with substance, distended and yellow-white coloured; adrenals dark-brown coloured; full bladder; pancreas showed diffuse reddening. The behaviour and the body-weight development of the surviving animals were normal during the follow-up period.

"The dissection of the animals killed at the end of the study showed no abnormalities.

"The oral median lethal dose (LD_{50}) for female rats is 3 400 mg/kg; the limits of confidence at p = 0.05 are 3 050 - 3 860 mg/kg body weight."

Conclusions: Acute oral LD_{50} for female rats: 3400 mg/kg confidence limits at p = 0.05 : 3050-3860 mg/kg. Oral toxicity category: III

Core Classification: Minimum

TOXICOLOGY BRANCH DATA REVIEW

Study Type: Acute dermal LD_{50}, male rats

Accession Number: 071795 (CI B:3)

MRID Number:

Sponsor: American Hoechst Corporation

Contracting Lab.: Hoechst Akliengesellschaft, report no. 593/82

Date: October 19, 1982

Test Material: ethyl-2-(4-(6-chlorobenzoxazolyloxy)-phenoxy)-propanoate, Fenoxaprop - ethyl , EC 12.5(Hoe 33171 OH EC037).

"A 40% emulsion in purified water (40 g/to 100 ml) was applied as a single-dose of 2 000 mg/kg body weight to the shorn backs of 6 male rats.

"The electrically shaven and intact skin of the back (approx. 30 cm^2) was treated, covered with aluminium foil (6 x 8 cm), which was fastened around the body of the animal with an elastic plaster bandage (Elastoplast®, 8 cm wide). After a 24 hour exposure period, the bandage was removed and the treated area of the skin washed with lukewarm water. After dermal application, the intoxication signs were recorded. During the 15-day follow-up period, the animals were weighed each week. The study animals were killed at the end of the follow-up period by CO_2 gas, dissected and submitted to macroscopic examination."

Results

"No animal died after application of 2 000 mg/kg body weight. 55 minutes after application, the treated animals exhibited drowsiness, and after 180 minutes a stilted gait and hyporeflexia. Twenty-four hours after application, the behaviour of all treated animals was once again normal. Dissection of the animals killed at the end of the study revealed no remarkable findings under macroscopic examination.

"The single-dose dermal LD_{50} for male rats is over 2 000 mg/kg body weight."

Conclusions: Acute dermal LD_{50} for male rats: more than 2000 mg/kg.

Dermal toxicity category: III

Core Classification: Minimum

003682

OL 43

TOXICOLOGY BRANCH DATA REVIEW

Study Type: Acute dermal LD_{50}, female rats

Accession Number: 071795 (CI B:4)

MRID Number:

Sponsor: American Hoechst Corporation

Contracting Lab.: Hoechst Akliengesellschaft, report no. 574/82

Date: October 19, 1982

Test Material: ethyl-2-(4-(6-chlorobenzoxazolyloxy)-phenoxy)-propanoate, Fenoxaprop - ethyl , EC 12.5(Hoe 33171 OH EC037).

"A 40% emulsion in purified water (40 g/to 100 ml) was manufactured and applied as a single-dose of 2 000 mg/kg body weight to the shorn backs of 6 female rats.

"The electrically shaven and intact skin of the back (approx. 30 cm^2) was treated, covered with aluminium foil (6 x 8 cm), which was fastened around the body of the animal with an elastic plaster bandage (Elastoplast®, 8 cm wide). After a 24 hour exposure period, the bandage was removed and the treated area of the skin washed with lukewarm water. After dermal application, the intoxication signs were recorded. During the 15-day follow-up period, the animals were weighed each week. The study animals were killed at the end of the follow-up period by CO_2 gas, dissected and submitted to macroscopic examination."

Results

"No animal died after application of 2 000 mg/kg body weight. Fifty minutes after application, the treated animals exhibited drowsiness, and after 180 minutes a stilted gait and hyporeflexia. Twenty-four hours after application, the behaviour of all treated animals was once again normal. Dissection of the animals killed at the end of the study revealed no remarkable findings under macroscopic examination.

"The single-dose dermal LD_{50} for female rats is over 2 000 mg/kg body weight."

Conclusions: Acute dermal LD_{50} for female rats: more than 2000 mg/kg.

Dermal toxicity category: III

Core Classification: Minimum

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003682

TOXICOLOGY BRANCH
DATA REVIEW

Study Type: 4-hour inhalation LC 50, male and female rats.

Accession Number: 071795 (C1 B:5)

MRID Number:

Sponsor: American Hoechst Corporation

Contracting Lab: Hoechst Aktiengesellschaft, report no. 26/82

Date: 12-8-82

Test Material: ethyl-2-(4-(6-chlorobenzoxazolyloxy)-phenoxy)-propanoate, Fenoxaprop-ethyl,EC, 120 g/l (Hoe 33171 O H EC036)

Protocol:

"Rats were exposed to known aerosol concentrations while kept in individual, cylindrical, plastic cages. The plastic tubes leading to the exposure cylinder were so fitted that only the animals' noses were inside the cylinder. The inhalation chamber itself was of 60 liter capacity. Compressed air was pumped at a pressure of 4 bar over an oil separation and absolute filter via a special pipe with a welded-in supply pipe for the test substance. The air supply at the nozzle outlet was maintained at a constant 800 l/hour by means of a calibrated air rotameter.

"Phenoxaprop EC was injected into the nozzle at a steady speed through a constant infusion apparatus. Primary aerosol formation took place in a 10 liter capacity four-neck round-bottom flask. Smaller aerosol particles (secondary aerosol) reached the inhalation chamber via a rising tube.

"At the base of the inhalation chamber was a suction device by which the aerosol could be suctioned off, via a cotton wool filter and sodium hydroxide solution, at a rate of 1 100 l/hour."

"Analytical investigations were carried out to determine the exact concentration in the inhalation chamber."

"Additionally, the aerosol concentration in the inhalation chamber was measured gravimetrically."

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"Aerosol particle - size distribution was determined by means of a particle counter (model 225 from Kratel KG, Gottingen."

'The study was carried out in four groups each of six males and six females. Inhalation time was four hours in each case.

Following inhalation, the animals were observed for a further 14 days and, with the exception of weekend, weighed daily and their behaviour noted."

"Lethally-poisoned animals were dissected and macroscopically examined. Remaining animals were killed under narcosis at the end of the follow-up period, dissected and similarly macroscopically evaluated."

Results:

"The inhalation study was carried out at a room temperature of 21 - 22°C and an air pressure of 972 - 1020 mbar. The temperature in the exposure cylinder was between 22.2 - and 25.3°C. During inhalation, 20.1 - 21.1% oxygen and 1 000 - 4 200 ppm carbon dioxide were measured. Carbon monoxide was not recorded.

"The rats showed the following concentration-related symptoms: narrowed lid openings. increased salivation, seromucous rhinorrhea, sneezing, irregular and jerky respiration, spasmodic respiration, noisy respiration (inspiratory whistling), balance disorders, ataxia, dizziness. squatting position, abdominal position and hyporeflexia.

"During the 14-day follow-up period, the surviving animals showed an initial weight loss and then gained weight regularly.

"Animal behaviour returned to normal within one to six days, depending on the concentration.

"At dissection the animals which had died intercurrently showed dark-red to black pulmonary foci, the animals which were killed at the end of the study showed no abnormal findings."

"No noteworthy findings were made macroscopically upon dissection of the animals killed at the end of the study."

Below are tabulated the percentage distributions of aerosol droplet size.

"Particle size intervals	Number in %			
	Group 1	Group 2	Group 3	Group 4
0.32 µm - 0.49 µm	20.4	16.4	17.4	16.4
0.50 µm - 1.49 µm	29.9	21.2	23.9	21.4
1.50 µm - 2.01 µm	16.5	13.5	17.4	12.2
2.02 µm - 2.99 µm	12.7	12.9	13.8	10.6
3.00 µm - 3.99 µm	5.0	7.9	7.4	6.2
4.00 µm - 4.99 µm	4.5	8.2	6.3	6.9
5.00 µm - 5.99 µm	2.6	5.5	3.8	5.2
6.00 µm	8.4	14.4	10.0	21.0

Below are tabulated the exposure concentrations and mortalities for each groups.

Group	Concentrations of Fenoxaprop EC (mg/m^3 air)		Mortalities (deaths/no. exposed)	
	Chemical Analysis	Gravimetric Method	Males	Females
1	1667	1993	0	0
2	3761	3352	4/6	2/6
3	4143	4771	6/6	0/6
4	5452	5531	6/6	6/6

There were no deaths at the 1667 mg/m^3 air concentration.

Conclusions:

The data are not adequate for establishing a specific LC 50. However, there were no male or female deaths at the 1667 mg/m^3 level after four hours exposure. This allows an inhalation toxicity category of II.

Core classification: Minimum

TOXICOLOGY BRANCH
DATA REVIEW

Study Type: Primary irritation, skin and eye, rabbits

Accession Number: 071795 (C1 B:6)

MRID Number:

Sponsor: American Hoechst Corporation

Contracting Lab: Hoechst Aktiengesellschaft, report no. 35/82

Date: 2-24-82

Test Material: ethyl-2-(4-(6-chlorobenzoxazolyloxy)-phenoxy)-propanoate, Fenoxaprop-ethyl,EC, 120 g/l (Hoe 33171 O H EC036)

I. PRIMARY SKIN IRRITATION STUDY

Protocol:

"The flank skin of 6 rabbits was shorn over an area of at least 6 x 3 cm with electric hair clippers. Half of this shorn area was additionally abraded with a scarifying clippper.

"Patches of cellulose gauze of Hansamed (R) wound bandage, 2.5 x 2.5 cm in size, were each covered with 0.5 ml of the emulsified concentrate Hoe 33171 O H EC036. They were then stuck onto the prepared sites and covered with an indifferent, impermeable polyethylene foil of 6 - 8 cm width. Finally, an elastic polyurethane chain-thread bandage was wrapped around the rump of each animal. (Permanent bandage K; manufacturer Lohmann). The exposure time was 24 hours. The first skin reactions were evaluated immediately after removal of the patches; further evaluations were made 48, 72, 96, 168 and 192 hours after the first start of the study."

"On the basis of the results, the primary irritation score was determined according to the method described in the U.S. Code of Federal Regulations, § 1500.41, as published in the Federal Register 38, No. 187,027019 (1973) see Table 1.

Results

"A primary irritation score of 1.4 was calculated after evaluating the effect of undiluted emulsified concentrate. In addition to the symptoms taken into account for the primary irritation score, the treated skin areas were dry, brittle, scaly (both fine and large), hardened, and showed both surface and deeper fissuring. In addition, skin detachment over a small area was observed. Eight days after the start of the study, all erythema and oedema had disappeared. According to the EPA guidelines, Hoe 33171 O H EC036 may thus be classified as slightly skin irritant."

Conclusions:

The verbally described skin irritation appears more severe than indicated by the reported numerical scoring. Based on the verbally expressed results a skin irritation category of II is appropriate.

Core Classification: Minimum

II. EYE IRRITATION STUDY:

Protocol:

The conjunctival sack of the left eye of each of nine rabbits was treated once with 0.1 ml of the fluid emulsified concentrate. The right eye of each animal acted as control. One minute after application, the treated eyes of three animals were washed out with physiological saline solution. The treated eyes of the six remaining animals were not washed out. The study was carried out according to the EPA guidelines as published in the Federal Register 43, No. 163, § 163.81-4, p.37359 (1978). The evaluation of the irritation effect was carried out with a magnifying glass, 1, 7, 24, 48, 72, 96, 168, 192, 216, 240, 264, 288, 360 and 384 hours after application. The 48- and 72-hour values were calculated after the additional instillation of a drop of sodium fluorescein, diluted 1:10,000 = 0.01 %.

Conclusions:

Data indicates that corneal opacity was seen in rabbit eyes after 7 days but not seen after 16 days.

Eye irritation category: ~~x~~ II w/E

Core Classification: Guideline

CONFIDENTIAL BUSINESS INFORMATION
DOES NOT CONTAIN
NATIONAL SECURITY INFORMATION (EO 12065)

003682

EPA: 68-01-6561
TASK: 47A
February 7, 1984

DATA EVALUATION RECORD

FENOXAPROP-ETHYL (HOE 33171 OH AS201)

Mutagenicity (Bacterial)

CITATION: Jung and Weingand. 1982. Study of the mutagenic potential of the compound HOE 33171 OH AS201 in strains of Salmonella typhimurium (Ames Test) and Escherichia coli. Report No. 432/82.

REVIEWED BY:

I. Cecil Felkner, Ph.D.
Project Scientist
Dynamac Corporation

Signature: Ira Cecil Felkner
Date: 2-7-84

Henry T. Appleton, Ph.D.
Program Manager
Dynamac Corporation

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Date: 2/7/84

APPROVED BY:

W. Thomas Edwards
EPA Scientist

Signature: W. Thomas Edwards
Date: 2-14-84

DATA EVALUATION RECORD 003682

STUDY TYPE: Mutagenicity (Bacterial).

CITATION: Jung and Weingand. 1982. Study of the mutagenic potential of the compound HOE 33171 OH AS201 in strains of Salmonella typhimurium (Ames Test) and Escherichia coli. Report No. 432/82.

ACCESSION NUMBER: 071787.

MRID NUMBER: Not available.

LABORATORY: Hoechst Aktiengesellschaft Pharma Research Toxicology, Department of Toxicology, Industrial Toxicology, Hoechst AG, Frankfurt/ Main.

TEST MATERIAL: The material used in this study was HOE 33171 OH AS201 which was a light brown crystalline powder. Purity of the material was not specified.

PROTOCOL:

Bacterial Strains: The bacteria used in this study included Salmonella typhimurium strains TA100, TA1535, TA1537, TA1538 and TA98 which are all auxotrophic (have a nutritional requirement) for histidine and which are collectively referred to as Ames' test strains; Escherichia coli WP2 uvrA, a tryptophan-requiring auxotroph was also used. Reversion of the bacteria to prototrophy (nutritional independence) is the principle upon which all of the assays in this study are based.

Preparation of Test Material: The test substance was dissolved in dimethylsulfoxide (DMSO) and applied at doses at 4, 20, 100, 500, 2,500, and 10,000 µg/plate in the cytotoxicity assay, with and without metabolic activation. In the mutagenicity assays, the same doses were used except 5,000 µg/plate was substituted for 10,000 µg as the highest test dose.

Methods: The liver homogenate fraction (S-9) was derived from Aroclor 1254-treated (500 mg/kg) male Sprague Dawley rats weighing 200 to 300 g each. Liver sections homogenized in KCl were centrifuged at 9,000 x g for 10 minutes and small aliquot volumes of the supernatant were separately frozen rapidly and stored at -80°C for not longer than 3 months. The

cofactors described by Ames et al.[1] plus the prepared S-9 fractions constituted the S-9 mix.

Mutagenesis Assays: The procedure used with Salmonella strains was essentially that described by Ames et al.[1]. In the case of E. coli WP2, the only difference was the composition of the top agar, in which 0.5 mM tryptophan was substituted for 1.0 mM histidine and 1.0 mM biotin. In the top agar were included 0.1 ml of the test material, 0.1 ml of an overnight culture of the appropriate bacterial tester strain, and 0.5 ml of S-9 mix (if required) or buffer. After incubation for 48 to 72 hours in the dark at 37°C, the revertant colonies were scored.

Dose range finding: A cytotoxicity test was performed by mixing 0.1 ml of different dilutions of the test material with 0.1 ml of a 10^{-6} dilution of overnight TA100 culture (the only strain used), and plating on histidine and biotin rich top agar (3 plates per dose). The ratio of values obtained with the solvent control and the treatment groups was recorded as the surviving fraction.

Control Chemicals: The positive control chemicals were sodium azide for TA100 and TA1535, 9-aminoacridine for TA1537, 2-nitrofluorene for TA98 and TA1538, and MNNG for WP2 uvrA in the absence of S-9 activation. In the presence of S-9 activation, benzo[a]pyrene and 2-aminoanthracene were used. DMSO was the solvent control.

RESULTS:

Sterility Checks: The test compound and S-9 mix were found to be free of bacterial contamination.

Cytotoxicity Test: At a dose range of 4 to 10,000 µg/plate, precipitation occurred at 500, 2,500 and 10,000 µg/plate in assays with or without S-9 activation. In the absence of metabolic activation, the background lawn of TA100 was reduced by 500, 2,500, and 10,000 µg/plate of the test material; the lawn of TA1535 was reduced at 10,000 µg/plate of the test substance. In the presence of S-9 activation, TA100 was inhibited at doses of 2,500 and 10,000 µg/plate. TA1535 and TA1537 were inhibited by 10,000 µg/plate of the test material.

Mutagenicity Assays: The test material, Hoe 33171 OH AS 201 did not induce revertants significantly above the control level in S. typhimurium strains TA98, TA1538, TA1537, TA1535, or TA100, nor did it increase the number of revertants in WP2 uvrA either with or without S-9 activation. All positive control chemicals used in the assays without S-9 activation were very active in mutation induction for all bacterial testers. In

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[1] Ames BN, McCann J and Yamasaki E. 1975. Mutat. Res. 31:347-364.

mutagenesis experiments with S-9 activation, the positive controls were usually effective in mutation induction, but there were some exceptions. Aminoanthracene induced 240 and 25 revertants in TA1535 in experiments 1 and 2, respectively, compared to a DMSO control average value of 11. With benzo[a]pyrene, average revertant values in TA1535 were 29 and 111 in experiments 1 and 2, respectively, compared to the control value of 11 revertants/plate.

DISCUSSION:

The authors concluded that the test material, Hoe 33171 OH AS 201 was not mutagenic in S. typhimurium or E. coli test systems either with or without metabolic activation at the dose levels investigated.

Our assessment is that Hoe 33171 OH AS 201 was not mutagenic under the conditions of the assays. Variability in response with benzo[a] pyrene and 9-aminoanthracene in S. typhimurium strain TA1535 was noted; i.e., approximately 10-fold differences at the same dose in two experiments, and in addition the higher response to benzo[a]pyrene was observed when response to aminoanthracene was low and vice versa. This observation is not unusual for this type of assay with the Ames strains, but it demonstrates that a "responsive/sensitive" bacterial strain relative to one positive control does not insure sensitivity relative to another positive control or, moreover, to the test material.

CONCLUSIONS:

Hoe 33171 OH AS201 was not mutagenic for the tester bacteria, either with or without S-9 activation, under the conditions of the assays at doses ranging from 4 to 5,000 µg/plate.

CORE CLASSIFICATION: Acceptable.

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CONFIDENTIAL BUSINESS INFORMATION
DOES NOT CONTAIN
NATIONAL SECURITY INFORMATION (EO 12065)

EPA: 68-01-6561
TASK: 47
February 7, 1984

DATA EVALUATION RECORD

FENOXAPROP-ETHYL (HOE 33171 OH AT 203)

Mutagenicity: Ames Assay

CITATION: Engelbart K and Scheerer M. 1979. Test for mutagenicity [Hoe 33171 OH AT 203] in bacteria strains in the absence and presence of a liver preparation. (An unpublished report no. 47/79 of Hoeschst AG).

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DATA EVALUATION RECORD

STUDY TYPE: Mutagenicity: Ames assay.

CITATION: Engelbart K and Scheerer M. 1979. Test for mutagenicity [Hoe 33171 OH AT 203] in bacteria strains in the absence and presence of a liver preparation. (An unpublished report no. 47/79 of Hoeschst AG).

ACCESSION NUMBER: 071787

MRID NUMBER: Not available.

LABORATORY: Arbeitsgruppe Molekularbiologie, Hoeschst Aktiengesellschaft, 6230 Frankfurt (M) 80 Postfach 80 0320, West Germany.

TEST MATERIAL: The test material was identified only as HOE 33171 OH AT 203. Its purity was not given in this study.

PROTOCOL:

Bacterial Strains: Salmonella typhimurium strains TA98, TA100, TA1535 and TA1537 were used in the reverse mutation assays described by Ames et al.[1]; however, strain TA1538 was not included. The bacteria were stored at -80°C, and recultivated in fresh nutrient broth at the time they were used.

Metabolic Activation: The S-9 fractions used for metabolic activation were prepared from a liver homogenate derived from Sprague-Dawley rats which were injected ip with 500 mg/kg of the polychlorinated biphenyl, Aroclor 1254, in a corn oil vehicle (dosage of 200 mg/ml) five days prior to sacrificed. Preparation of the S-9 fraction and S-9 mix was according to the procedure of Ames et al.[1] The amount of S-9 used in the mix was varied from 0.04 to 0.1 ml because the exact dosage was determined from pretests using known positive and negative control compounds.

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[1] Ames BN, McCann J, and Yamasaki E. 1975. Mutation Res 31:347-364.

Control Compounds: For the negative solvent control, dimethylsulfoxide (DMSO) was used both with and without S-9 activation. In the absence of S-9, procarbazin-HCl (5 µg/plate) was used for strains TA98 and TA100, and streptozotocin (10 µg/plate) was used with strain TA1537. In the absence of S-9, 2-aminoanthracene (5 µg/plate) was used for all of the four Salmonella auxotrophs, and it was also assayed without S-9 to demonstrate that metabolism was required to generate an active mutagenic form.

Preparation of Test Material: HOE 33171 OH AT 203 was diluted in DMSO and delivered to the assay plates at the final concentrations of 4, 20, 100, 500, and 1500 µg/plate in the absence of S-9 and at 4, 20, 100, 500, and 2500 µg/plate in the presence of S-9. According to the protocol some positive control or test compounds were prepared in water or ethanol, but specified in each special case, however, there was no mention of the use of either solvent in the results section.

Procedure: The authors stated that "the methods used are published in detail by Ames et al." (see Reference 1). Briefly, however, test strains TA98, TA100, TA1535, and TA1537 were exposed to dosages of HOE 33171 OH AT 203 at 2500 to 4 µg per plate, with and without metabolic activation. The plates were incubated for 48 hours at 37°C, after which the revertant colonies were scored.

RESULTS:

In the absence of S-9 activation, the number of revertant colonies for TA98, TA100, TA1535, and TA1537 were not significantly different from the solvent control numbers in four replicates plates with the tester strains at concentration HOE 33171 OH AT 203 concentrations ranging from 4 to 1500 µg/plate. The response of TA98 to 5 µg/plate of Procarbazin-HCl was the induction of 3300 revertants (average of 2 plates) compared to 16.8 revertants (average of 4 plates) in the solvent control. For TA100, the solvent control gave 81.3 revertants/plate (average of 4 plates) compared to 2040 revertants per plate after treatment with 5 µg/plate of Procarbazin-HCl. For strain TA1535, the solvent control had 18.3 revertants per plate (average of 4 plates) versus 3900 revertants per plate after treatment with 10 µg/plate of Streptozotocin. For strain TA1537, the solvent control plates averaged 4.8 revertants per plate (average of 4 plates) compared to 4300 revertants/plate (1 plate) after treatment with 9-aminoacridine.

In the presence of S-9 activation, the number of revertant colonies for TA98, TA100, TA1535, and TA1537 were not significantly different from the solvent control numbers in four replicate plates with the tester strains at HOE 33171 OH AT 203 concentrations ranging from 4 to 2500 µg per plate. For each tester strain, the response to 5 µg/plate 2-aminoanthracene without and with S-9 activation was, respectively, TA98, 2350 and 24 (average of 2 plates); TA100, 3350 and 79 (average of 2 plates); TA1535, 168.5 and 11 (average of 2 plates); and TA1537, 131.5 and 11.5 (average of 2 plates). The average of 4 plates with the DMSO control gave revertants colony per plate numbers of 23.5 for TA98; 78.3 for TA100; 8.5 for TA1535; and 6.8 for TA1537.

DISCUSSION:

The authors concluded that the test material, HOE 33171 OH AT 203 did not induce a mutagenic response "neither in the presence nor absence of rat liver preparation in the dosage range of 2500 µg to 4 µg."

Our assessment of the data is in agreement with the authors except that the highest dosage of test material in the absence of S-9 was 1500 µg/plate. In addition, strain TA1538 was not included in the study. Since the authors stated that the assay was as described by Ames et al.[1], this mutant would ordinarily be included. However, the use of both TA98 and TA1538 is not considered to be necessary since both strains contain the identical His^- gene defect (hisD3052). The purity and batch number of HOE 33171 OH AT 203 were not provided in this study.

CONCLUSIONS:

HOE 33171 OH AT 203 (undefined purity) did not induce mutations in Salmonella typhimurium strains TA98, TA100, TA1535 and TA1537 under the conditions of the assay.

CORE CLASSIFICATION: ~~Unacceptable in the present form. The study can be upgraded to acceptable if a description of the test material, including the purity is provided.~~

accepted.
H.T.C.

CONFIDENTIAL BUSINESS INFORMATION
- DOES NOT CONTAIN
NATIONAL SECURITY INFORMATION (EO 12065)

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EPA: 68-01-6561
TASK: 47
February 7, 1984

DATA EVALUATION RECORD

FENOXAPROP-ETHYL (HOE 33171 OH AT 204)

Mutagenicity (Mouse Micronucleus)

CITATION: Leist, Weigand, and Kramer. 1982. Testing of HOE 33171 OH AT 204 for mutagenicity after oral administration to NMRI mice - micronucleus test. (Report No. 49/82. Unpublished reports dated 12/11/81, 11/17/81 and 2/24/82 submitted by Hoechst Aktiengesellschaft, Pharmaceuticals, Research Toxicology).

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DATA EVALUATION RECORD

STUDY TYPE: Mutagenicity.

CITATION: Leist, Weigand, and Kramer. 1982. Testing of HOE 33171 OH AT 204 for mutagenicity after oral administration to NMRI mice - micronucleus test. (Report No. 49/82. Unpublished reports dated 12/11/81, 11/17/81 and 2/24/82 submitted by Hoechst Aktiengesellschaft, Pharmaceuticals, Research Toxicology).

ACCESSION NUMBER: 071787.

MRID NUMBER: Not available.

LABORATORY: Pharma Research Toxicology, Hoechst AG, Postfach 80 03 20 6230, Frankfurt/Main 80, West Germany.

TEST MATERIAL: The test material was identified as HOE 33171 OH AT 204 which contained "93 percent HOE 33171 in accordance with Ref. No. 01711 dated 01/10/1981 (Prof. Keller)." The material consisted of light brown crystals.

PROTOCOL:

Species: The species was mouse, NMRI strain Hoe: NMRKF (SPF 71).

Preparation of Test Material: The test material was weighed into a glass beaker, blended with sesame oil and mixed for about one minute with an Ultra-Turrax, "washed into a 25- ml volumetic flash, topped up to the calibration mark with sesame oil and stirred with a magnetic agitator for approximately three minutes." The concentration of HOE 33171 was 180 mg/ml (4,500 mg of HOE 33171 in a total volume of 25 ml). Dilutions of this stock preparation were made daily with sesame oil at a ratio of 1:10 in 25 ml volumetic flasks.

Positive Control: To 100 mg of Endoxan [cyclophosphamide] in an injection vial, 5 ml of distilled water were added. This mixture was shaken until it produced a clear stock solution. Daily dilutions were prepared by adding 2 ml of this 2 percent Endoxan stock solution to 6 ml of distilled water and mixing. The daily prepared diluted solutions were used for dosing the positive control mice.

Dosing and Route of Administration: The vehicle negative control, Endoxan positive control, and test material were administered orally by stomach tube to 5 animals of each sex per group as indicated:

Group No.	Volume (ml/kg)	Concentration (g/100 ml vehicle)	Dosage (mg/kg)
1	10 (vehicle)	0	0
2	10 (HOE 33171)	0.18	18
3	10 (HOE 33171)	1.8	180
4	10 (HOE 33171)	18.0	1800
5	20 (Endoxan)	0.5	100

Bone Marrow Preparation:

According to the authors "six hours after the second administration, the animals were killed with CO_2 gas."

The procedures used to obtain bone marrow samples and to prepare slides for examination was "in accordance with W. Schmid, 1976." Both femurs of each animal were removed, freed of muscle tissue, opened and the marrow contents flushed with 1 ml of calf serum into siliconized centrifuge tubes containing 5 ml of fetal calf serum. After centrifugation for five minutes at 1,000 x g, the supernatant was almost completely removed "and the sediment carefully blended with the aid of a Pasteur pipette." A drop of this marrow cell suspension was spread onto a glass slide, air dried for about 24 hours, and stained by the May-Grunwald/Giemsa procedure, rinsed with distilled water, blotted and the back of the slide cleaned with methanol. The slide(s) was placed in xylene for 5 minutes and embidded in Entellan. The number of slides prepared for each animal was not reported.

Evaluation of Slides: The number of micronuclei in 2,000 polychromatic erythrocytes (PCEs) were determined for each of 5 animals of both sexes. As a control, the number of mature erythrocytes containing micronuclei was noted. The slides were codified and examined by cytologists. The number of PCEs with micronuclei per 2,000 PCEs and the number of monocytes with micronuclei per 1000 normocytes were scored and statistically evaluated. The ratio of PCEs to normochromic erythrocytes was likewise evaluated. Statistical evaluations for differences between treated and control samples "were carried out separately for each sex in accordance with the Nemenyi method[1]", with all statistics being performed using a computer program developed by the Applied Mathematics Dept. of Hoechst AG. A 95 percent significant level was used.

[1] No reference was given for the Nemenyi method.

RESULTS:

The authors stated that two male animals from the 1,800 mg/kg group displayed "closed palpebral fissures 3-4 hours after the second administration" and that "In all other animals, the behavior was unaffected by the test compound." The data supporting these observations, however, were not presented.

The authors also stated that no HOE 33171 OH AT 204 doses caused changes in numbers of micronuclei (MN) per 2000 PCEs or the ratio of PCEs to normocytes relative to controls, but that cyclophosphamide induced statistically significant increases in PCEs with MN in animals of both sexes.

Negative control males averaged 14 MN/2000 PCEs; negative control females averaged 4.4 MN/2,000 PCEs; negative control males averaged 1.8 MN/1000 normocytes; and negative control females averaged 2.0 MN/1000 normocytes. The ratios of PCEs to normocytes in negative control male and female mice, respectively averaged 714.2:1000 and 913:1000. By contrast Endoxan-treated males averaged 154.8 MN/2,000 PCEs; Endoxan-treated females averaged 149.4 MN/2,000 PCEs; Endoxan-treated males averaged 1.8 MN/1000 normocytes; and Endoxan treated females averaged 2.4 MN/1000 normocytes. The ratios of PCEs to normocytes in Endoxan-treated male and female mice, respectively, averaged 526.6:1000 and 459:1000. Based on these findings, our assessment is that the test had adequate sensitivity to detect micronuclei induction and depression of the hematopoietic process. Although, statistical significant values were not identified in the report, it was stated that "Cyclophosphamide induced a marked, statistically significant increase in polychromatic erythrocytes with micronuclei in the animals of both sexes."

DISCUSSION:

The authors concluded that HOE 33171 OH AT 204, at the dosages used, did not change significantly the numbers of PCEs and normocytes with micronuclei from the values obtained in control animals of either sex. In addition, they concluded that the test material did not affect the ratios of PCEs to normocytes in either sex.

From our assessment of the data, we conclude that the authors have interpreted their data correctly. However, by observing the marrow taken from animals only 6 hours after the administration of the second dose, any affect from that dose would most likely have been missed. There should have been 8-12 hours allowed after the last mitosis, the time required for the nucleus to be expelled, so as to properly score the micronuclei after treatment. The protocol of Schmid[1] has this shortcoming, which is

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[1] Schmid, W. 1975. Mutat. Res. 31:9-15.

corrected by the procedure of Heddle and Salamone[2] using sacrifice times of 48, 72, and 96 hr after the last treatment with two doses separated by 24 hours. In addition, it is recommended that the highest dose should be 80 percent of the LD_{50}, but the authors failed to report the basis for selecting the dosages used in this study.

CONCLUSIONS:

Under the conditions of the assay, HOE 33171 OH AT 204 did not induce micronuclei at a level statistically higher than the negative controls in NMRI mice at dosages of 18, 180, or 1800 mg/kg. However, the basis of dose selection and sacrifice time after administration of the last dose require justification. In addition, the statistical values and the reference for the method used should have been incorporated into the report.

CLASSIFICATION: The report of this study is unacceptable in its present form. The dosage levels and timing and number of sampling intervals should be justified and the statistical treatment of the data should be documented. It would then be possible to reevaluate the report.

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[2] Heddle, JA and Salamone MF. 1981. In Stich, HF and San RHC. Short-Term Tests for Chemical Carcinogens, Springer-Verlag, NY/Heidelberg/Berlin, 1981, pp. 244-251.

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February 7, 1984

DATA EVALUATION RECORD

FENOXAPROP-ETHYL (HOE 33171 OH AS 201)

Mutagenicity (Gene Conversion)

CITATION: Mellano D. and Berruto G. 1982. Study of the mutagenic activity of the compound HOE 33171 ~~OH~~ AS 201: Gene conversion-DNA repair test (Study Number M-416).

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DATA EVALUATION RECORD

STUDY TYPE: Mutagenicity.

CITATION: Mellano D. and Berruto G. 1982. Study of the mutagenic activity of the compound HOE 33171 OH AS 201: Gene conversion-DNA repair test (Study Number M-416).

ACCESSION NUMBER: 071787.

MRID NUMBER: Not available.

LABORATORY: Instituto di Ricerche Biomediche "Antonine Marxer" S.p.A, Casella Postale 226, 10015 Ivrea.

TEST MATERIAL: The test material was identified as "HOE 33171 OH AS 201 (this denomination also includes the batch number)," a brownish crystalline material of 94.0 percent purity. The sponsor stated that it was stable at room temperature (20°C) and in DMSO regardless of the concentration.

PROTOCOL:

Yeast Strain: The yeast, Saccharomyces cerevisiae D4 was used in this study. If was supplied by "Laboratorio di Mutagenesi e Differenziamento" of CNR Pisa. It is a doubly heterozygotic heteroallelic diploid strain with respect to mutations in genes for adenine and tryptophan biosynthesis.

Control Materials: The positive controls were endoxan (258 and 375 µg/ml) in the assays with S-9 and methylmethanesulfonate (84.5 µg/ml) in the assays without S-9 activation. The negative control was dimethylsulfoxide (DMSO) at 27.5 mg/ml. Methylmethanesulfonate (MMS) was prepared in deionized water at 3.38 mg/ml and 0.1 ml of this solution was added to 3.9 ml of the treatment mixture. Endoxan was dissolved at a concentration of 1.035 mg/ml or 1.5 mg/ml (CP solution) and 1 ml of the CP solution was added to 3.9 ml of the activated treatment mixture.

Preparation of the Test Material: A sample of 149.5 mg of Fenopropethyl (HOE 33171 OH AS 201) was dissolved in dimethylsulfoxide (DMSO), and further diluted in DMSO to obtain concentrations of 40, 20, 10, and 5 mg/ml. The test material was then diluted from these stock solutions in DMSO and used to treat the test organism at concentrations of 125, 250, 500, and 1000 µg/ml.

Media: Liquid growth medium consisted of glucose (20 g), yeast extract (10 g), peptone (20 g), adenine sulfate (40 mg), tryptophan (40 mg) in 1 liter of water. Plating media were as follows: complete medium, consisting of 20 g glucose, 10 g yeast extract, 20 g peptone and 15 g agar added to one liter of deionized water. Selective medium with adenine, consisting of MM B2 (Biolife), 15 g agar and 40 mg adenine sulfate added to one liter of deionized water. Selective medium with tryptophan consisted of 22.2 g MM B2 (Biolife), 15 g agar and 40 mg tryptophan added to one liter of deionized water. All media were sterilized at 1.0 atm. for 15 min. The agar media was brought to 60°C after sterilization and poured into sterile plastic Petri plates (9 cm diameter).

PROCEDURE:

Spontaneous Frequency Assays: Precultures of S. cerevisiae D4 were inoculated by transferring several silical granules with a sterile loop into 100 ml of liquid growth medium. The preculture was shaken in a water bath at 32°C for 16 hr, after which one ml was diluted 10^{-5} and 0.05 ml of the resultant dilution inoculated into each of 10 screw-cap tubes that contained 10 ml of liquid growth medium. These yeast cultures were shaken at 32°C for 48 hr, and after this incubation period aliquots were taken, "appropriately diluted and a sufficient volume of the suspension was placed on the selective growth medium and on the complete growth medium plates (4 plates per type of medium)." The suspension was usually diluted at 10^{-5} onto complete medium for viable counts and 10^{-1} onto selective medium for assessing convertants. After incubation at 32°C for 3 to 4 days, the plates were scored. The ratio of colonies that developed on minimal agar with adenine or with tryptophan to colonies on complete agar medium gave the spontaneous frequencies of trp 5^+ and ade 2^+ convertants, respectively.

Test Culture Preparation: The test culture was prepared by inoculating 0.3 ml of the stored culture into 100 ml of culture medium and incubating with shaking at 32°C for 16 hr. After incubation, the culture was centrifuged at 1000 x g for 10 min., the supernatant decanted, and the cells resuspended in saline to a concentration of about 5×10^8 (as determined by microscopy using a hemocytometer).

S-9 Mix Preparation: Livers were obtained from adult male Sprague-Dawley rats (Charles River CD) weighing 150 to 250 g and which had previously received a single ip dose of Aroclor 1254 (in a corn oil solution of 200 mg/ml) at 500 mg/kg, livers were obtained. The livers were homogenized for 30 sec. at 40°C in a 0.15 M KCl solution and centrifuged at 9,000 x g for 10 min. in a refrigerated centrifuge. The S-9 fractions were divided into aliquots, frozen and stored at -70°C. The protein concentration of S-9 was determined using the biuret method[1] and its activity checked (conversion of a promutagen to a mutagen) by an assay

[1] Weichselbaum TE. 1946. Amer. J. Clin. Path. 16:40.

with three Salmonella strains, TA1538, TA98, and TA100, using 2-Aminofluorene (2-AF) as the substrate. The S-9 mix contained 0.4 ml of S-9, 0.6 ml of 0.1 M phosphate buffer (pH 7.4), 20 µl of 0.5 M $MgCl_2$ and 2 M KCl, 2.9 mg of glucose-6-phosphate, and 1.5 mg of NADP.

Gene Conversion Assays: In the assay without metabolic activation, 0.1 ml of the test material, DMSO or MMS was added to 2.9 ml of phosphate buffer and 1 ml of the yeast cell suspension (~ 500 x 10^6 cells/ml). In the assays with metabolic activation 0.1 ml of the test material, DMSO or CP solution was added to 1.9 ml of phosphate buffer, 1 ml of S-9 mix and 1 ml of the yeast cell suspension (~ 500 x 10^6 cells/ml). The treatment mixtures with and without S-9 were then incubated for 4 hr. at 35°C in a horizontally shaking water bath.

After incubation, aliquots of the treatment mixtures were diluted in physiological saline and plated onto complete medium or selective medium plates, exactly as described for the spontaneous frequency assays. The plates were incubated for 3 to 4 days and convertant colonies were then scored.

The convertant frequency for the trp 5 gene or the ade 2 gene was calculated as a ratio of the mean number of colonies growing on selective media to the mean number per 10^4 colonies growing on complete medium. The relative survival at various doses was calculated by multiplying the survival at each dose by 100 and dividing by the control survival.

Statistical Evaluation: The Chi-square method was used to compare the test material data to that for the negative control and positive control data.

RESULTS:

The convertant frequencies for the trp 5 gene and for the ade 2 gene in S. cerevisiae without metabolic activation were 2.65×10^{-5} and 2.86×10^{-5}, respectively, in the negative controls compared with frequencies of 22.34×10^{-5} for trp 5 and 24.87×10^{-5} for ade 2 after treatment with 84.5 µg/ml of MMS. After treatment with HOE 33171 without S-9 the frequencies for trp 5 and ade 2 genes were not significantly different from the negative controls. The highest convertant frequencies calculated for the HOE 33171 concentration range of 125 to 1000 µg/ml was 2.65×10^{-5} for trp 5 and 2.86×10^{-5} for ade 2; both responses were obtained by treating the yeast cells at a dose of 125 µg/ml for 4 hr. The convertant frequencies for the positive control (MMS) treatments were significantly elevated ($p<0.001$) whereas no level of HOE 33171 treatment increased the convertant frequency at a significant level ($p>0.05$).

The convertant frequencies for the trp 5 gene and for the ade 2 gene in S. cerevisiae D4 with S-9 activation were 2.55×10^{-5} and 2.92×10^{-5}, respectively, in the negative controls compared to frequencies of 10×10^{-5} for trp 5 and 11.9×10^{-5} for ade 2 after treatment with endoxan (CP). After treatment with HOE 33171 with S-9 activation, the

frequencies for the trp 5 and ade 2 genes were not significantly different from the negative controls ($p>0.05$). The highest convertant frequencies calculated for the HOE 33171 concentration range of 125 to 1,000 µg/ml were 2.55×10^{-5} for trp 5 and 2.92×10^{-5} for ade 2; both responses were obtained by treating S. cerevisiae D4 at 125 µg/ml for 4 hr.

After treatment with the test material in the absence of S-9 the percent survival for S. cerevisiae D4 ranged from 69.93 (treatment at 125 µg/ml) to 49.91 (treatment at 1,000 µg/ml). Treatment with MMS, the positive control, for 4 hr resulted in 60.34 percent survival for this yeast. After treatment with the test material in the presence of S-9 the percent survival for S. cerevisiae D4 ranged from 99.01 (treatment at 125 µg/ml) to 82.71 (treatment at 1,000 µg/ml).

Treatment with the CP solution resulted in a 78.38 percent survival.

DISCUSSION:

The authors concluded that treatment of S. cerevisiae D4 with HOE 33171 OH AS 201 at concentrations up to 1,000 µg/ml in the absence or presence of S-9 did not induce significant increases in gene conversion.

Our assessment of the data is that the authors made a correct evaluation of their results and that the assay had an appropriate level of sensitivity because the positive controls gave good response. The authors also verified the spontaneous level of gene conversion for both markers. The yeast survival level in the absence of S-9 was 42.91 percent, indicating that the highest concentration of HOE 33171 was adequate. However, the yeast survival level in the presence of S-9 was 88.71, indicating that a higher concentration of the test material could have been used. We recommend treatment resulting in approximately 37 percent survival because at this level, the average cell in the population receives one lethal hit. By comparing the survival after MMS treatment (60.34 percent) to that after CP treatment (78.38 percent) and the conversion frequencies e.g., 22.34×10^{-5} versus 10×10^{5}, one sees that treatment which decreases survival may also increase the gene conversion frequency. The authors should have given their reasons, e.g., solubility limitations, for not treating the tester yeasts at higher test material concentrations. Also, the authors should have been more specific in identifying which component of the HOE 33171 OH AS 201 designation gives reference to the batch number.

CONCLUSIONS:

Under the conditions of the assays, HOE 33171 OH AS 201 did not induce gene conversion to a level significantly elevated above the solvent controls, either with or without S-9 activation at dosages up to 1,000 µg/ml.

CLASSIFICATION: Acceptable.

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DATA EVALUATION RECORD

FENOXAPROP-ETHYL (HOE 33171 OH AS 201)

Mutagenicity

CITATION: Mellano D. and Berruto G. 1982. Study of the mutagenic activity "in vitro" of the compound HOE 33171 OH AS 201 with Schizosaccharomyces pombe. (An unpublished report of Study No. M 417 prepared by RBM Institute for Hoeschst AG).

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DATA EVALUATION RECORD

STUDY TYPE: Mutagenicity.

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ACCESSION NUMBER: 071787.

MRID NUMBER: Not available.

LABORATORY: RBM laboratories. Instituto di Ricerche Biomediche "Antoine Marxer" S.p.A. Casella, Postate 226, 10015, Ivrea.

TEST MATERIAL: The test material was identified as "HOE 33171 OH AS 201 (this denomination also includes the batch number" and was 94.0 percent pure. It was in the form of brown crystals; the sponsor stated that it was stable at "20° C and in DMSO regardless of the concentration."

PROTOCOL:

Yeast Strain: The yeast strain used for this study was Schizosaccharomyces pombe (SP ade6-60/rad10-198, h-), a haploid mutant yeast developing red pigmented colonies while mutated colonies are white. The original supplier of this strain was "Laboratio di Mutagenesi e Diffrenziamento" of the CNR (Pisa).

Preparation of the Test Material: A sample of 118 mg of HOE 33171 OH AS 201 was dissolved in dimethylsulfoxide (DMSO), and further diluted in DMSO to obtain working stock solutions of 40, 20, 10, 5 mg/ml. The test material was then diluted from these stock solutions in DMSO and used to treat the test organisms in liquid growth medium at doses of 125, 250, 500, and 1000 µg/ml.

METHODS

Controls: After initial solubilization in DMSO, working stock solutions of methylmethanesulfonate (MMS) at 3.38 mg/ml and DMNA (not further identified but presumed to be dimethylnitrosamine) at 15 mg/ml were prepared in deionized water. DMSO was used as a negative control and was present in a stock solution at a concentration of 27.5 mg/ml. In the assays, the solvent control, DMSO was further diluted 1:40, MMS at 84.5 µg/ml was the positive control without S-9 activation, and DMNA at 375 µg/ml was the positive control with S-9 activation.

Metabolic Activation: The liver homogenate fraction (S-9) was prepared from the livers of adult male Sprague-Dawley rats weighing 150 to 250 g which had received an ip dose of 500 mg/kg of Aroclor 1254. Liver slices were homogenized in 0.15M KCl (solution: tissue ratio of 3:1) at 4° C. The homogenate was centrifuged at 9,000 x g in a refrigerated centrifuge and the supernatant (S-9) divided into aliquots that were deep frozen and stored at -70° C. The protein concentration was determined by the biuret method[1], and the activation capacity of the S-9 fraction was assayed using 2-aminofluorene (2-AF) and S. typhimurium strains TA98, TA100 and TA1538 in a system for detecting mutation induction. The S-9 mix, consisted of 0.4 ml of S-9 (protein concentration determined from assays), 0.6 ml of 0.01 M phosphate buffer (pH 7.4), 20 µl of 0.5 M $MgCl_2$ and 2M KCl, 2.9 mg of glucose-6-phosphate and 1.5 mg of NADP.

Mutagenesis Assays: The incubation mixtures for the assays without metabolic activation were as follows: negative control, 29 ml of phosphate buffer, 0.1 ml of DMSO, and a yeast cell suspention (~ 550 x 10^6).

The incubation mixtures for the assays with S-9 activation were as follows: negative control, 1.9 ml of phosphate buffer, 0.1 ml of DMSO, and 1.0 ml of a yeast cell suspension (~ 550 x 10^6); positive control, 1.9 ml of phosphate buffer, 0.1 ml of DMNA solution, 1.0 ml of S-9 mix and 1.0 ml of a yeast cell suspension (~ 550 x 10^6); test material, 1.9 ml of phosphate buffer, 1.0 ml of S-9 mix and 1.0 ml of a yeast cell suspension (~ 550 x 10^6).

All mixtures were incubated with horizontal shaking at 35 °C for 4 hours in a water bath. After incubation and dilution in physiologic saline to give ~90,000 cells/ml, an aliquot was further diluted 1:10. From the most concentrated suspension, 0.1 ml was poured into a test tube of soft agar held at 50 °C; the contents were mixed and plated (14 plates designated per determination). From the 1:10 diluted solution aliquots were plated (4 plates designated Px) by the same procedure. All plates were incubated for 5 days at 32 °C and scored for white mutant colonies (P) and for total colonies (Px).

The ratio of mutated colonies to total colonies gave the mutation frequency and survival was calculated by multiplying the total number of colonies by 100 and dividing the resultant number by the number of cells plated. Relative survival at various doses was determined by multiplying survival at each dose by 100 and dividing by the control survival value.

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[1] Weichselbaum, TE. 1946. Amer. J. Clin. Path. 1bi40.

Statistical Evaluation: The Chi-square method was used to compare the spontaneous mutation frequency in the negative control with the mutation frequencies in the positive controls and in the test material mixtures at various concentrations.

RESULTS:

Survival of Schizosaccharomyces pombe after treatment with HOE 33171 OH AS 201 in the absence of S-9 activation ranged from 90.58 percent at a dose of 125 µg/ml to 51.95 at a dose of 1000 µg/ml. Survival after treatment with MMS at 84.5 µg/ml was 48.28 percent. The mutation frequencies x 10^{-4} were as follows: Negative control (0.87); HOE 33171 at 125 µg/ml (0.77), at 250 µg/ml (0.43), at 500 µg/ml (0.48) and at 1000 µg/ml (0.33); Positive control, MMS (14.1). None of the HOE 33171 treatments resulted in a statistically significant increase at $p>0.05$, however, the value for MMS was significant at $p<0.001$.

DISCUSSION:

The authors concluded that HOE 33171 OH AS 201 did not induce significant increases in gene mutation of Sch. pombe either in the absence or presence of S-9 activation.

Our assessment indicates that under the conditions of this assay, HOE 33171/OH AS 201 did not induce white colonies as a consequence of a mutation in any of the five genes controling adenine biosynthesis. However, if solubility is not a limiting factor, a dose of the test material should be used which results in a survival of 37 percent in order to insure one lethal hit per dosed cell. The test material and the positive controls were not given this level of dosing and although mutation frequencies were significantly increased in the positive controls it cannot be assumed that the optimum conditions for mutagenesis were met. However, the authors demonstrated that mutagenesis was induced by the positive controls in the tester yeast strains under the experimental conditions used.

CONCLUSIONS:
The test materal, HOE 33171 OH AS 201 (94.0 percent pure), was not mutagenic for Sch. pombe ade 6-60/rad 10-198, h- under the conditions of the assay with or without S-9 activation at doses up to 1,000 µg/ml.

CLASSIFICATION: Acceptable.