12-15-86 PP0-439 TR-5632

005632

DEC 15 1986

MEMORANDUM

SUBJECT: Fenoxaprop-ethyl (WHIP, Acclaim)

FROM: Thomas Edwards, Pharmacologist
Hazard Evaluation Division (TS-769) W Thomas Edwards 12-10-86

TO: Richard Moutfort
Registration Division (TS-767)

THRU: Marcia Van Gemert Ph. D., Section Head M. VanGemert 12 10 86
Review Section III

and

Theodore Farber Ph D, Chief
Toxicology Branch, HED (TS-769)

Chemical: Fenoxaprop-ethyl (WHIP, Acclaim) W.F. Barnes 12/10/86.

Caswell No.: 431 C

Accession Nos.: 263030-263037

EPA I D No.: 8340-EG / 6F3316

Record No. : 174545/174546

Project No. : 2048

Requested Actions: Review Hoechst response including new data and comment on adequacy for registration. The memorandum to which the responses were made is attached.

Comments:

Following and numerically listed are EPA conclusions. Each is followed by its Hoechst Response or a reference to that response which in turn is followed by our current comments.

1. CONCLUSION: The acute studies for inhalation toxicity and eye irritation must be replaced.

AMERICAN HOECHST'S RESPONSE: It was explained that solid Fenoxaprop-ethyl when passed through a laboratory pulverizer, for generating inhalable particles, a waxy material resulted. Also it was explained that the waxy solid was not suitable for eye irritation testing.

2. CONCLUSION: The mutagenicity studies (Micronucleus,UDS) must be replaced.

AMERICAN HOECHST'S RESPONSE: See attachment.

COMMENT : The explanations concerning the mutagenicity studies are accepted. Results are negative.

3. CONCLUSION: The two hepatic enzyme studies (rat and mouse) included assays made after one year only. The studies showed dosage related lipid effects. There are also indications in short term studies, including dermal and inhalation studies, that effects on lipid enzymes and other enzymes are considerably larger than shown by longer term studies. Clarification of the nature and extent of these is needed. It is suggested that protocols be submitted to EPA for consideration before studies are begun. Determinations of other blood and urine changes should be included. Also, as appropriate, histopathology should be performed.

AMERICAN HOECHST'S RESPONSE: See attachment.

COMMENT: There appears to be little disagreement about blood lipid effects of fenoxaprop-ethyl, but there remains disagreement about importance of "pharmacological" effects.

The argument that "pharmacological" effects are not of toxicological concern is not agreed with. Such changes are effects and not to be ignored.
It is of some concern that (as stated in American Hoechst's response) that Fenoxapropethyl caused an increase of blood lipids in mice rather than a decrease as found in rats. However, neither change is wanted. Tolerance calculations are to be based on the NOEL from the most sensitive species tested.

Justification for not using the results obtained from testing a particular species could, of course, be supported if it were proven that because of differences in metabolism the effects would not be found in humans.

"Pharmacological" effects from short-term studies should not be neglected.

All data received will be used in our considerations.

005632

1. CONCLUSION: The NOEL (15 mg/cu m) for repeated dose inhalation is equivocal. It is noted that histopathology was not conducted for the animals given 15mg/cu m or for recovery animals at any dosage level. Clarification is needed. Additional pathology and study of lipid metabolism is needed.

   AMERICAN HOECHST'S RESPONSE: See attachment.

   COMMENT: The principle concern for this study was with the relationship of phenoxaprop-ethyl treatment to lipid effects. There was also interest in any differences in effects resulting from the different routes of absorption. Comments on lipid effects are made in Item 3. above.

   Nevertheless, after considering Hoechst's response, it is agreed that this study is acceptable and that the NOEL for Repeated Dose Inhalation is 15 mg/cu m.

5. CONCLUSION: A NOEL was not determined in the Repeated Dose Dermal study. Also, the data suggests that dermal absorption may be greater than gastrointestinal absorption.

   AMERICAN HOECHST'S RESPONSE: See attachment.

   COMMENT: EPA agrees with the American Hoechst response that neither the study data nor additional submitted information show that dermal exposure is more toxic than oral exposure. Nevertheless, a Repeated Dose Dermal NOEL was not found. Comments on relevance of lipid effects are expressed under Item 3 above.

   Pending the approval of an adequate study, product labeling must require the wearing of protective clothing by applicators.

6. CONCLUSION: The ADI based tentatively on dog NOEL of 3 ppm (0.075 mg/kg/day) with a safety factor of 100 is 0.0008 mg/kg/day. Unpublished tox. approved plus the current action utilize 2.45 % of the ADI.

   AMERICAN HOECHST'S RESPONSE: See attachment.

   COMMENT: It is agreed that for the reasons stated the NOEL should be 15 ppm instead of 3 ppm. The ADI will therefore be based on another of the three major studies. The 2- Generation Study has the NOEL 5 ppm (0.25 mg/kg/day). With a safety factor of 100, the ADI is 0.0025 mg/kg. The percent of the ADI utilized by the current action is 0.30.

005632

7. CONCLUSION: The fetal NOEL was not determined in the 2-generation study (LDT, 5 ppm). Decreased survival and body weight of offsprings and significant changes in kidney and liver weights were found in F2a and F2b litters. Decreased survival of offsprings were significant at all dosage levels but apparently not dose related. A new study must be submitted prior to issuance of permanent tolerances.

AMERICAN HOECHST'S RESPONSE: See attachment.

COMMENT: The replacement study is adequate. A DER is attachei

No reproductive effects were found.

Maternal and pup NOELs : 5 ppm
LEL : 30 ppm (reduced body weights in Fla pups, and reduced blood lipids in parents).

8. CONCLUSION: Consideration of need for repeating the mouse oncogenicity is attached as a separate presentation.

AMERICAN HOECHST'S RESPONSE: See attachment.

COMMENT: Increased cholesterol,total lipids and increased liver weights are not adequate determinants for setting a MTD. As indicated in the 32 day feeding study a suitable MTD would be somewhat less than 315 ppm, at which dosage level, kidney necrosis was found in females.

The highest dosage tested in the mouse Oncogenicity study (40 ppm is approximately 1/8 th of this 315 ppm dose.

The Toxicology Branch's Draft Position Paper Maximum Tolerated Dose (MTD) is attached. When this paper was used to assess the need for repeating the chronic mouse study, the first 6 of 7 levels indicated that the highest dosage tested was inadequate for use as an MTD. Level 7 allowed for a conclusion to be based on comparison of a Margin of Safety (calculated from the Highest Dosage Tested) with estimated human exposure. Calculations according to Level 7 follow.

Highest Dose Tested : 40 ppm = 6 mg/kg/day

Assumed worse case exposure : 0.05 ppm in 100% of diet (in 1.5 kg of food).
0.05 mg/kg of food x 1.5 kg = 0,075 mg
0.075 mg/day / 60 kg mans weight =1.25 x 10^{-3} mg/kg/day

Assumed MOS ratio : highest dosage tested / daily intake =
6 mg/kg/day / 1.25 x 10^{-3} = 4800

005632

Therefore the mouse study is considered to be acceptable as long as residue remains very low, eg. 0.05 ppm or less. If the company wishes to have increased residue levels, the whole question of the adequacy of the study will have to be reassessed.

TERATOGENICITY CONCLUSION: Teratogenic NOEL: 50 mg/kg
LEL: 200 mg/kg (diphragmatic hernias in rabbits)

AMERICAN HOECHST'S RESPONSE: See attachment.

Comment: We do not agree with company response concerning diaphragmatic hernias. No evidence presented demonstrated that the finding of hernias at 200 mg/kg level was erroneous. The diaphragmatic hernias found in pups of the 200 mg/kg group are considered to be the result of developmental effects caused by fenoxaprop-ethyl treatment rather than due to secondary effects related to maternal debility.

Maternal Toxicity: NOEL, 12.5 mg/kg/day
LEL, 50 mg/kg/day (decreased food consumption and body weight)

Embryofetal Toxicity: NOEL, 50 mg/kg/day
LEL, 200 mg/kg/day (reduced body weight, increased incidence of [illegible], diaphragmatic hernias)

Ratio of Maternal to Embryofetal Toxicity = 12.5 / 50 = 0.25 for the rabbit study.

Margin of safety calculations were included in our memorandum of 2-13-86, which is attached. These are unchanged.

Also, it is noted that the rat study referred to in the same memorandum indicated a Maternal to embryofetal ratio of 32 mg/kg/day / 32 mg/kg/day = 1

In summary,

1. The NOEL from a Repeated Dermal Toxicity study is still lacking. Pending the approval of an adequate study, the labeling must require the wearing of protective clothing by mixer loaders and applicators. Goggles are also required.

2. Note that the Mouse Oncogenicity Study is acceptable only as long as the residue remains below 0.05 ppm.

005632

TOXICOLOGY BRANCH DATA REVIEW

Study Type: 2-Generation Reproduction, Rat.

Accession Number: 263030

Project No.: 2048

Record No.: 174545/174546.

Sponsor: American Hoechst Corp.

Contracting Lab: Research and Consulting Corp., No. A32781.

Date: 2-20-86

Test Material: Fenoxaprop-ethyl

Caswell No.: 431C

EPA Evaluation Review. W Thomas Edwards (Thomas Edwards) 12-9-86 (Date)

Review Section Approval. M. VanGemert (Marcia Van Gemert, Ph D) 12/9/86 (Date)

Protocol: See procedures attached.

WISTAR/HAN Rat (Kfm: WIST, outbred, SPF Quality) from KFM, Kleintierfarm Madoerin AG, Ch 4414 Fuellinsdorf/Switzerland were used.

Dosage levels were the following 0, 5, 30, and 180 ppm. Each group consisted of 30 males and 30 females.

Other aspects of procedure are in attached MATERIALS AND METHODS.

Introduction:

This study was submitted in response to EPA comments on the previous Reproduction Study, numbered A30806/A31073. A new study was needed because a NOEL for fetal toxicity had not been found. Decreased survival of offsprings and changes in kidney and liver weights in F2a and F2b litters were found at lowest dosage level.

In this study the dosage levels were the same as in the previous study (5, 30, and 180ppm).

This study is considered to have been submitted to furnish information for clarifing the previous study. No histopathology information was included.

Results:

No fenoxaprop-ethyl related survival effects were found in parent or pup groups.

No clinical signs of toxicity were seen.

No body weight or food consumption effects on parent groups were found.

Reproduction parameters were not affected.

Increased parental liver and kidney weights and relative weights were observed at 180 ppm in males of the 180 ppm dosage groups, especially in the F1 generation. See attached data.

Dose related statistically significant effects on blood lipids (total lipids, and cholesterol) were found in F1 parents at all dosage levels. At the 5 ppm level this effect could be considered borderline. See attached data. Also, alkaline phosphatase was increased in pups. The increase was significant in all groups 3 and 4, except in F2b. These changes were possibly related to lipid metabolism. PRotein reduction in groups 3 and 4 were not large but possibly related to kidney effects.

On day 21 post partum, somewhat decreased pup decreased body weights were found at the 30 ppm in the F1a group and in all 180 ppm groups.

Increased liver and kidney weights were found in pups at the 180 ppm dosage level but not at lower levels. Data is attached. Also, several organ weights and organ weight ratios were decreased in high dosage groups. These included lungs and spleen. These effects are considered secondary to debility of the animals.

Conclusions:

Unlike the previous study, this study found no effects on pup survival or pup organ weights in the 5 ppm groups. It must be concluded that this difference between studies was due to the reported Viral infection in animals of the first study.

Reproduction parameters were not affected (180 ppm HDT).

Maternal Toxicity, NOEL: 5 ppm.
LEL: 30 ppm (blood lipid effects at 30 ppm, increased liver and kidney weights at 180 ppm). Histopathology was not reported for this study. Increased incidences of nephrocalcinosis at the 180 ppm level was reported in the previous study.

Developmental Toxicity, NOEL: 5 ppm.
LEL: 30 ppm (reduced body weights in Fla pups).

Reduced body weights were found in all groups of the next higher dosage, 180 ppm).

Since this study was intended only to determine reproductive effects,the maternal lipid effect may not be relevant unless related to reduced fetal body weight.

Core Classification:

Minimum.

Fenoxaprop-ethyl Toxicology Reviews

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

Pages 9 through 70 are not included. The pages contain detailed methods/results submitted by the pesticide registrant.

REVIEWER 1

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

005632

004963

OFFICE OF
PESTICIDES AND TOXIC SUBSTANC

MEMORANDUM

SUBJECT: Fenoxaprop-ethyl, WHIP, ACCLAIM, Hoe 033171

FROM: Thomas Edwards, Pharmacologist
Hazard Evaluation Division (TS-769)

TO: Richard Mountfort
Registration Division (TS-767)

THRU: Clint Skinner, Section Chief
Review Section III

and

Theodore Farber, Chief
Toxicology Branch, HED (TS-769)

Chemical: Fenoxaprop-ethyl

Caswell No.:431C

EPA Registration Nos.:8340-EUP-7, 3G2940, 8340-EUP-8, 4G3035, 8340-EG, 8340-EE, 6F3316, 8340-RI.

Accession Nos.: 255848-255858, 256666-256667, 256763, 073961-073973, 258947-258972.

Requested Actions: Review data and comment on adequacy for EUP and for registration.

Comments:

Attached is a summary of status of submitted or needed testing studies. One-liners and DERs are also attached.

Conclusions

1. The acute studies for inhalation toxicity and eye irritation must be replaced.

2. The mutagenicity studies (Micronucleus, UDS) must be replaced.

004963

005632

3. The two hepatic enzyme studies (rat and mouse) included assays made after one year only. The studies showed dosage related lipid effects. There are also indications in short term studies, including dermal and inhalation studies, that effects on lipid enzymes and other enzymes is considerably larger than shown by longer term studies. Clarification of the nature and extent of these effects is needed. It is suggested protocols be submitted to EPA for suggestion before studies are begun. Determinations of other blood and urine changes should be included. Also, as appropriate, histopathology should be performed.

4. The NOEL (15 mg/cu m) for repeated dose inhalation is equivocal. It is noted that histopathology was not conducted for the animals given 15mg/cu m or for recovery animals at any dosage level. Clarificationis needed additional pathology and study of lipid metabolism is need.

5. A NOEL was not demonstrated in the repeat dermal study. Also the data suggests that dermal absorption may be greater than gastrointestinal absorption.

6. The ADI based tentatively on dog NOEL of 3ppm (0.075 mg/kg/day) with a safety factor of 100 is ~~TAS~~ 0.0008 mg/kg/day. Unpublished Tox. appraisal plus the current action ratio is 2.45% of the ADI.

7. A fetal NOEL was not determined in the 2- generation study (LDT, 5 ppm). Decreased survival and body weight of offsprings and significant changes in kidney and liver weights were found F2a and F2b litters. Decreased survival of offsprings were significant at all dosage levels but apparently not dose related. A new study must be submitted prior to issuance of permanent tolerances.

8. Consideration of need for repeating the mouse oncogenicity study is attached as a separate presentation.

In summary, it appears from the conclusions that the data received ~~perhaps~~ marginally justifies the issuance of an EUP, or an extension of a temporary exemption, but not a permanent tolerance or full registration.

The various data gaps need to be filled and equivocal findings clarified before further decisions can be made.

Free Standing Summary of Currant Status of Submitted Studies usefull for Registration of Fenoxyoxaprop-ethyl, Whip, Acclaim, Hoe 033171,Technical

Acute oral toxicity

LD 50 (rat):2357 mg/kg. Toxicity category, III. Core guideline.

Acute dermal toxicity

LD 50 (rabbit): >1000 mg/kg. Toxicity category, II. Core minimum. A category indicating less hazard might be justified if a study using a higher exposure is submitted.

Acute inhalation toxicity

INVALID study only.

Eye irritation

INVALID study only.

Dermal sensitation.

Not sensitizing. Core minimum.

42-Day repeated dose inhalation toxicity, rat

NOEL: 15 mg/cu m. (provisionally acepted)
LEL: 75 mg/cu m. (Clinical changes found in both sexes and ketonuria in males indicate disturbances in lipid metabolism). Other studies may clarify concerns.

Repeated dose dermal toxicity,rat (30 days)

A NOEL was mot found. LEL was 2 mg/kg (LDT). Clinical changes were found in both sexes. ketonurea in males was found at all dosage levels. Sodium levels for high dosage males did not return to normal levels during the 4 weeks recovery period.

Ames test

Not mutagenic. Acceptable.

Chromasome Abberation

Not mutagenic. Acceptable.

Micronucleus test

Unacceptable. A replacement is required.

Unscheduled DNA synthesis

Unacceptable. A replacement is needed.

Hepatic enzyme assays in rat and mouse

Assays were only made after 12 months. Some indication of dose related lipid effects were found. Earlier assays are needed.

Chronic toxicity, rat (6-,12-,24-months)

NOEL: 30ppm. LEL:180ppm (decreased serum cholesterol especially in males).

Oncogenicity, rat (28-months)

Not oncogenic at 180 ppm (HDT).

Oncogenicity/chronic toxicity, mouse (24)

A MTD was not found at 40ppm (HDT). Toxicity LEL:more than 40 ppm. The study was not adequate for oncogenicity determination because a minimum effective dose was not found.

See attached statement of consideration of need for repeating this study because of no MTD.

Chronic toxicity, dog (2-years)

NOEL: 3 ppm. LEL: 15 ppm (reduced body weight).

Metabolic studies, rat

A 10 mg/kg single dose, labled in the chlorophenyl ring, was excreted rapidly. Biphasic. Greater storage was in fat. 98% was eliminated in 7 days from males. 100%, females.

Similar results were obtained when labeled dose followed 14 daily doses of unlabled material. 3 to 4% remained in tissue after 168 hours. Mostly in kidney, liver, blood, and fat. Other tissues contained less than 0.1 ppm.

When in a 24 months study 2 rats were sacrificed at 6, 12, 18, and 24 months,it was found that dose related increases were not sex related. Lack of increase with time suggested steady state metabolism.

These studies are acceptable.

Metabolism studies pregnant, rat, rabbit, and monkey

The radioactivity in fetus was small compared to other tissues.

Faster tissue absorption in rat than in mouse was found. There was slightly faster elimination in rat than in mouse. Monkey data was not comparable. Different dosing. Only one monkey (which was not killed) was used.
Acceptible supplimental.
These studies are acceptable.

Tissue residues

Dosage related residues were found. Accumulations with time or or sex related differences were not found.

Metabolism studies pregnant, rat, rabbit, and monkey

The radioactivity in fetus was small compared to other tissues.

Faster tissue absorption in rat than in mouse was found. There was slightly faster elimination in rat than in mouse. Monkey data was not comparable. Different dosing. Only one monkey (which was not killed) was used.
Acceptible supplimental.

2-Generation reproduction, rat

Maternal NOEL: 30 ppm. LEL: 180 ppm (increased liver and kidney weight; nephrocalcinosis)

Fetal NOEL: Not determined. LEL: 5 ppm (LDT) (decreased survival and body weight of offsprings and significant changes in kidney and liver weights for F2a and F2b litters.

Teratogenicity, rabbit

Maternal NOEL: 12.5 mg/kg. LEL: 50 mg/kg (decreased food consumption and weight gain).
Fetal NOEL: 50 mg/kg. LEL: 200 mg/kg (increased incidence of rib anomalies. decreased fetal weight).

Teratogenicity NOEL: 50 mg/kg. LEL: 200 mg/kg (diaphragmatic hernia).

The teratogenic margin of safety based the NOEL of 50 mg/kg/ day for one serving of rice per day was calculated to be 740,000. One serving of soybean oil, 260,000. If it were assumed that 4 servings would be eaten per day, the MOS's would be 180,000 for rice and 66,000 for soybean oil.

Teratogenicity, rat

Maternal NOEL: 32 mg/kg. LEL: 100 mg/kg (reduced body weight increase).

Fetal NOEL: 32 mg/kg. LEL: 100 mg/kg (delayed ossification and slightly impaired growth).

Teratogenicity NOEL: more than 100 mg/kg (HDT).

The maternal LEL of 100 mg/kg appeared to be borderline but was accepted, both fetal and maternal effect (even though slight) were noted at 100 mg/kg.

Teratogenicity, monkey

Fetotoxicity and maternal toxicity LEL's: 10 mg/kg (LTD), the only dosage having an adequate number of survivors. No controlls were used. Teratogenicity was not adequately accertainable. Supplimental (not required).

Teratogenicity,dermal application, rabbit.

Teratogenicity, fetotoxity, and maternal toxicity NOEL: 1000 mg/kg (HDT). The amount of absorption was not determined.

Supplimental (not required).

Teratogenicity, dermal application, rat

Teratogenicity, fetotoxicity, and maternal toxicity NOEL: 1000 mg/kg (HDT). The amount of absorption was not determined.

Supplimental (not required).

Consideration of the Need for repeating the Mouse Oncogenicity Study

Because no MTD was found, there is concern relating to the adequacy of the study. In compliance with a proposed branch policy, the following conciderations are offered.

There were no 90 day mouse feeding studies for determining an MTD.

A 32 day study mouse study reported increased absolute and relative liver weights at all dosages. Lesions in liver and kidney were found at 80 ppm the lowest dosage tested.

A 30 day mouse study study reported increased liver and kidney weight gains at 10 ppm.

There were two other rodent (rat) studies (24 and 28 months). The LEL was found to be 9 mg/kg/day whereas the HDT for mouse was 6 mg/kg/day. The rat LEL was 50% higher than the mouse HDT.

Only two types of acceptable mutation tests were available, the Ames test and a chromosome abberation test. These were negative, but other mutagenicity testing is required.

No structural analogue was shown by a search of CIS sources to be an oncogen.

Following are Margin of Safety calculations for diet exposure, which are usefull in Toxicology Branch consideration of the need for additional testing.

$$MOS = \frac{\text{NOEL (mg/kg/day)}}{\text{daily intake (mg/kg/day)}}$$

Residue in rice = 0.02
Single serving portion of rice = 202 gms.
Residue in soybean oil = 0.05
Single serving portion of soybean oil = 227.
HDT used as NOEL = 40 ppm = 6 mg/kg/day.

Daily intake of rice (asuming one serving per day).

$$\frac{202 \times 0.05}{1000 \times 60} = 0.00017 \text{ mg/kg/day for a 60 kg person.}$$

$$\text{MOS for rice} = \frac{6}{0.00017} \quad 35{,}000$$

Daily intake of soybean oil (assuming one serving per day).

$$\frac{227 \times 0.05}{1000 \times 60} = .000189 \text{ mg/kg/day for a 60 kg person.}$$

$$\text{MOS for soybean oil} = \frac{6}{0.000189} = 31{,}000$$

If it were assumed that a person would eat 4 servings each day instead of one, the calculated margin of safety would be 8,700 instead of 35,000 for rice and 7,900 instead of 31,000 for soybeans.

Although concern remains regarding the adequacy of the oncogenicity testing in mouse because of lack of MTD, the above calculations suggest that the margin of safety for dietary exposure is large.

Calculations of applicator exposure have been requested but not yet received.

A final determination regarding the requirement for an additional mouse oncogenicity study needs to be deferred until additional mutagenicity data and applicator exposure studies are reviewed.

005632

File last updated 4/5/85

DRAFT

NOEL change not recorded ss

mg/kg			mg/kg/day	mg/day(60kg)
0.075	3.0	100	0.0008	0.0450

unpublished,Tox approved 332940,433035

CROP	Tolerance	Food Factor	mg/day(1.5kg)
Soybeans (oil)(143)	0.03	0.92	0.00069
Rice(137)	0.20	0.55	0.00017

TPI	TMRC	% ADI
0.0450 mg/day(60kg)	0.0009 mg/day(1.5kg)	1.90

Current Action 332940,433035 [Rev Sec 3]

CROP	Tolerance	Food Factor	mg/day(1.5kg)
Soybeans (oil)(143)	0.030	0.92	0.00090
Rice(137)	0.030	0.55	0.00025

TPI	TMRC	% ADI
0.0450 mg/day(60kg)	0.0011 mg/day(1.5kg)	2.45

BEST AVAILABLE COPY

Response to EPA Review of Submitted Studies

This is being written in response to the EPA comments on the full toxicology package submitted in support of the registration of HOE 33171. The specific EPA conclusions are listed in a letter (No. 00493) from Mr. Thomas Edwards to Mr. Richard Montfort.

EPA Conclusion 1:

The acute studies for inhalation toxicity and eye irritation must be replaced.

Response: - Inhalation Toxicity (Doc. No. A 24752; Accession No. 071787) A response to the EPA review of this study was submitted on December 7, 1984. No EPA review of this response has been received at this time.

The active ingredient is a brown crystalline solid material when it is frozen. It was intended that a dust inhalation study be conducted. When the active ingredient was put through the laboratory pulverizer, in order to generate inhalible particle sizes, the compound turned into a waxy material. Therefore dust inhalation was not feasible.

In the inhalation study a 5% solution of the active ingredient in ethanol: polyethyleneglycol 400 (1:1) was used. At the higher concentrations tested the active ingredient recrystallized on the spray nozzle. On the basis of the present study, it is possible to correlate analytically measured concentrations and observed toxicity. Thus the inhalation study

should be accepted as submitted since technical limitations required modification of the study design. In addition, this study is in agreement with OECD-regulations and was conducted before the 1982 EPA guidelines became effective.

Under the described study conditions, the four hour LC_{50} for HOE 033171 active ingredient was greater than 511 mg/m^3 air. At this highest possible concentration only one out of 12 animals died (Doc. No. A 24752; Accession No. 071787).

The acute inhalation study with the emulsifiable concentrate (1 EC = 90 g/L) in rats resulted in a LC_{50} value which is equivalent to 783 mg active ingredient/m^3 air. The determination of the LC_{50}-value was based on a clear dose-response relationship (Doc. No. A 27490; Accession No. 071795). Thus the determined values for the active ingredient which were generated in both studies are in a comparable range.

This finding is supported additionally by the fact that in the subchronic inhalation study in rats with HOE 033171 active ingredient, one female out of 12 animals died after the first exposure at a dose of 727 mg/m^3 (Doc. No. A 29699; Accession No. 255851). It should be taken into consideration, however, that the latter study was performed in a different laboratory and not under exactly identical conditions as in the first two studies.

The facts support the position that the inhalation study with the active ingredient of HOE 033171 is valid since the acute toxicity was reproducible under different conditions.

Response: - Eye irritation (Doc. No. A 24688; Accession No. 071787)
A response to the EPA review of this study was submitted on December 7, 1984. No EPA review of this response has been received at this time.

According to our laboratory experience it is technically impossible to use the original test substance, since it turns to a waxy material at room temperature and very quickly solidifies or forms clumps. Due to the fact that a non-homogeneous particle size range exists, the direct application of this material into the conjunctival sac is not desirable. Therefore, it was necessary to apply the moistened test substance, using some droplets of polyethyleneglycol 400, i.e. 0.3 ml vehicle and 500 mg HOE 033171 per eye, equivalent to a concentration of 67%. The use of a moistened substance, represents an advantage because the material is more homogeneous than a solid article. The evaluation of the data indicate that HOE 033171 active ingredient is a slight irritant. This is a scientifical valid statement. The eye irritancy test should be accepted as submitted since technical limitations required modifications of the study design. In addition, this study procedure is in agreement with OECD-regulations and was conducted before the 1982 EPA guidelines became effective.

EPA Conclusion 2:

EPA stated that the mutagenicity studies (Micronucleus Test and UDS) must be replaced.

Response: - Micronucleus Study (Doc. No. A 29691; Accession No. 258849) In the case of the micronucleus study, no justification for this request was submitted by the EPA. Based on the EPA review presented on April 11, 1984 the report (Doc. No. A 23744; Accession No. 071787) was rewritten, reevaluated and resubmitted. In the second version of the report, dated September 19, 1984 (Doc No. A 29691) the dose levels and the timing and number of sampling intervals were justified and the statistical treatment of the data were documented at the request of the EPA.

At the time the micronucleus test was performed the investigation was based on the scientific knowledge available (W. Schmidt, Mut. Res. 31, 9 - 15, 1975). With respect to a possible influence on the hematopoietic cycle, the data presented do not give any indication of such an effect since the ratio of polychromatic to normochromatic erythrocytes was not influenced by the test substance even though sublethal doses were given. Since two different sampling times were introduced, namely 6 hours after the second dosing or 30 hours after the first dosing of mice, the study should be judged as valid. The conclusion that no chromosomal mutations occurred has a high predictive value.

At the time the study was conducted (1981) the recommendations as stated in the EPA Gene-Tox Program (Mut. Res. 123, 61 - 118, 1983) were not available. Therefore, the micronucleus test should be considered as an appropriate study. Furthermore, an acceptable chromosome aberration analysis in human lymphocyte culture in vitro was submitted to the Agency, indicating no mutagenic potential of HOE 033171 (see Doc. No. A 26277; Accession No. 255849).

We were requested by the Agency to re-write a micronucleus report on our compound HOE 39866. This study was performed using the same protocol and methods as with HOE 33171. We re-wrote the report in the same manner as we did for HOE 33171. This re-written report was accepted by the Agency.

Response: - UDS-Test (Doc. No. A 24582; Accession No. 255849)
Referring to the UDS-Test, we agree with the criticism of EPA, that only a minimal number of 3 dose-groups was used. However, based on the data presented, no (not even marginal) effects on UDS were demonstrated at the highest or second highest dose. Therefore, a dose-dependent effect cannot be expected and there is no further need for clarification in order to draw conclusions relative to a dose-related effect. The EPA Gene-Tox Program (Mut. Res. 123, 363 - 41, 1983) describes test procedures, but at the time the study with HOE 033171 was performed (October 1982) these recommendations were not yet available for consideration.

With respect to the discussion of limited sensitivity, due to the "high background levels of radioactive label" we would like to comment as follows:

- The ability of the test system to detect DNA-damaging activity by the positive control substances methylmethanesulfonate (MMS; -S9) and cyclophosphamide (CP, +S9) was clearly demonstrated.

- Different statistical evaluations of the data (Student's t-test or Duncan's test) confirmed the presented evaluation, which indicated that the three doses of the test substance did not significantly increase the induction of UDS as compared with the solvent control.

- Considering the individual data (triplicate analyses) in the control and dose groups both with and without metabolic activation, there was no indication of any genotoxic effect, because there was no increase in cpm-values in the treatment groups. Therefore, it seems unlikely that the postulated "subtle effects" which may be induced by the test substance could not be detected due to the fact that the background levels of radioactive label in the negative control group were relatively high.

In general we feel that the present study has to be judged as scientifically valid, although cultured HeLa cells were used. The authors of the report clearly indicated that in the method used, hydroxyurea suppresses normal semiconservative DNA replication without appreciably inhibiting the synthesis of a new DNA, consequent to repair processes, i.e.

the absolute and relative repair synthesis can clearly be assessed. Therefore, it must be accepted that the authors conclusion is accurate and that under the test conditions the test substance HOE 033171 can be considered not to induce effects that cause increased cell repair processes. Additionally, there is no scientific justification to use diploid human cells (e.g. WI-38) instead of HeLa cells, because there is no proof that both test systems will respond in a different way.

The EPA Data Requirements for Pesticide Registration (40 CFR 158) indicate that a battery of mutagenicity tests must include tests appropriate to address gene mutations, structural chromosomal aberrations and other genotoxic effects. The table below summarizes the types and results of mutagenicity tests performed with HOE 33171:

End Point	Assay	Results	EPA Review	Accession No.
Gene Mutation				
	Ames	Negative	Acceptable	071787
	Ames	Negative	Acceptable	071787
	S. pombe	Negative	Acceptable	071787
Chromosomal Aberration				
	Human Lymphocytes	Negative	Acceptable	255849
	Micronucleus	Negative	Reserved	071787
	Micronucleus	Negative	Not Acceptable	255849
Other Mechanisms of Mutagenicity				
	UDS	Negative	Not Acceptable	255849
	Gene Conversion-DNA repair	Negative	Acceptable	071787

Each assay type performed is considered by the EPA to be acceptable and representative for the end point measured (Pesticide Assessment Guidelines, Subdivision F, pages 148-151).

Based on these results and our arguments for the UDS and micronucleus tests we do not feel that any additional significant scientific information will be gained by performing more mutagenicity tests. In addition it should be noted that rat and mouse oncogenicity studies have been performed and are absolutely negative in respect to oncogenicity.

EPA Conclusion 3:

EPA required clarification on the mode of action of HOE 033171, especially the effect on lipid metabolism in short-term studies in comparison to chronic studies.

Response:

HOE 33171 affects the lipid metabolism in rats and mice but not in dogs. In rats there is a decrease in cholesterol and therefore total lipids. In mice the pattern is exactly opposite, that is, an increase in both parameters. It is our belief that this change is related to the pharmacodynamic activity of the test material. Table 1 summarizes the effects of HOE 33171 in rats. At high doses (>315 ppm; 32 days) the lipids were decreased, liver and kidney weights increased, and alkaline phosphatase increased. Histopathology of the liver indicated hepatocellular hypertrophy with a fine-granulated eosinophilic staining of the cytoplasm. Necrosis was also observed at these high doses. In all of the rat studies performed there was no pathologic lesion in the kidney though the kidney weight was increased at the higher doses.

A clear pattern of effects on lipid metabolism can be seen in the 32 day, 90 day and chronic/carcinogenicity studies. In all cases the cholesterol and total lipids are decreased. At 26 weeks in the chronic study an effect on lipids was observed at all dose levels. As the study progressed the magnitude of the effect lessened until 106 weeks where only the high dose was effective. In all cases effects on lipids are seen at doses that do not produce liver damage as indicated by liver weight, alkaline phosphatase or hepatocellular hypertrophy. These changes are reversible as indicated by the recovery periods in the 13 week oral study and the subchronic inhalation and dermal studies (see Tables 2 & 3).

Based on the pattern of effects in all studies it is concluded that HOE 33171 pharmacodynamically affects lipid metabolism. At extreme doses there is hepatocellular hypertrophy and in some cases necrosis. At lower doses there is an initial hepatomegaly but as time progresses the liver returns to normal until at the end of lifetime exposure there is actually a statistically significant decrease in liver weight. It is clear from all of these studies that consumption of HOE 33171 has no clear chronic adverse health effects. The animal is fully able to adapt to HOE 33171.

The EPA has requested additional work to be done on the hepatic enzymes. This work is included in this submission (Tab C-8). These studies show

that HOE 33171 at doses up to 20 ppm does not induce the liver metabolism, deplete glutathione or enhance peroxisomal proliferation.

We feel that we have adequately addressed the question of effects on lipid metabolism. Under normal circumstances serum total lipids and cholesterol would not be measured in any of the toxicity studies. These are not required in Subdivision F of the Pesticide Assessment Guidelines. The special studies we performed to determine enzyme induction and peroxisomal proliferation are also not required by the Guidelines. These measurements were made to help elucidate the possible role they may have played in the toxic response. As the data indicate, affects on lipid metabolism have no long term toxic consequences. Further investigation of this phenomenon will not affect the NOEL's in the studies. Any additional information which may be gained by future study would be difficult to include in any risk assessments. The fact that HOE 33171 lowers the lipids could be considered a health advantage rather than a disadvantage or risk.

The health profile of lipid lowering agents is well known. The reviewer is directed to a review by Cohen and Grosso (Fd. Cosmet. Toxicol. Vol 19, pp 585 to 605, 1981) for a summary of these agents. Another recent paper of interest is "Preclinical Toxicology Studies with the Lipid-Regulating Agent Gencadiol" (Fitzgerald et al, Fundamental and Applied Toxicology Vol 6, pp 520 to 531, 1986). This compound has essentially the same pattern of effects in the rat as HOE 33171. The paper concludes that the liver alterations "reflect compensatory manifestations of altered metabolism related to the lipid-regulating activity of the compound."

EPA Conclusion 4.

The NOEL (15 mg/cu m) for repeated dose inhalation is equivocal. It is noted that the histopathology was not conducted for the animals given 15 mg/cu m or for recovery animals at any dosage level. Clarifications needed additional pathology and study of lipid metabolism is needed.

Response:

Table 2 summarizes the pertinent parameters in the 6 week inhalation study and 13 week feeding study. It is evident that the effects on lipid metabolism after inhalation exposure are the same as those after dosing in the feed. The effects on lipid metabolism are clearly due to the pharmacodynamic lipid lowering activity of HOE 33171. The four week recovery group in the inhalation study shows that the effects are reversible. The effects on lipid metabolism are discussed in detail in the response to EPA conclusion number 3 above. We do not feel that it is necessary to do any additional work on lipid metabolism in the inhalation study. It is evident that the reviewer had no access to the other studies on HOE 33171.

We agree with the EPA that no histopathology was performed in the 15 mg/cu m group or in the recovery animals. However there is no indication of any toxicity in any of the animals to warrant histopathology. It is clear from all of the rat studies that effects on cholesterol and total lipids are observed at doses below those which produce histopathological changes. It is also evident that hepatocellular hypertrophy is only seen microscopically where the liver weight is

increased. In the 15 mg/cu m group there are no changes in lipids, alkaline phosphatase or organ weights. Since histopathologic examination of the next higher concentration (73 mg/cu m) did not reveal any pathologic changes, it is not necessary to evaluate the lower concentration (15 mg/cu m). No changes were evident in any of the parameters evaluated after the recovery period. Therefore, histopathologic evaluation of these recovery animals is not needed.

In conclusion, we feel that NOEL of 15 mg/cu m has been identified. Inhalation exposure does not result in a pattern of toxicity different from that after oral exposure. Additional histopathologic information is not justified and would not be expected to change the NOEL.

EPA Conclusions. 5.

A NOEL was not demonstrated in the repeat dermal study. Also the data suggests that dermal absorption may be greater than gastrointestinal absorption.

Response:

The effects observed in the dermal study were the same as those in the 13 week feeding study. Table 3 illustrates the response pattern. Essentially the only changes were decreases in total lipids and cholesterol and an increased liver weight. No pathologic changes were observed in the liver or kidney. All changes reversed during the four week recovery period.

These lipid changes are clearly due to the pharmacodynamic action of HOE 33171. They have no long term toxic consequences. A review of the effects of HOE 33171 on lipid metabolism is presented above in the response to EPA conclusion number 3. It is evident that the reviewer of this study was not aware of the other studies performed with HOE 33171.

Based on the results of this study it is clear that 20 mg/kg is the NOEL. Dermal exposure does not result in pattern of toxicity different from oral exposure. There were no organ weight effects or histopathologic changes at this dose. The statement that dermal absorption may be greater tnan gastrointestinal absorption is based on a typographical error in the review. The lowest dose tested was 20 mg/kg not 2 mg/kg as stated in the review. The comparable NOEL for the 13 week feeding study was 20 ppm or about 2 mg/kg. Thus the ratio of oral to dermal is 2:20 suggesting about 10% is absorbed. Dermal penetration studies with the formulated material indicate that greater than 50% of the material is absorbed (Tab No. C-10).

EPA Conclusion 6:

The ADI based tentatively on dog NOEL of 3 ppm (0.075 mg/kg/day) with a safety factor of 100 is 0.0008 mg/kg/day.

Response:

We do not agree with the reviewers' conclusion that 3 ppm is the NOEL for the two year dog study. The only effect observed in this study was reduced body weight in the males and females at 75 ppm. The reviewer contends that

there is a significant effect on body weight in the males at 15 ppm and therefore the NOEL is 3 ppm. We do not agree with this conclusion. Evaluation of the body weight data at study initiation and termination indicates that the 15 ppm males weighed less than the other animals at initiation. The effect at the end of the study is due to reduced initial body weights. Table 4 outlines these figures. Statistical evaluation of the body weight change over the term of the study indicates that only the 75 ppm group is significantly different from the control. Based on this evaluation of the data the NOEL is 15 ppm.

The male dogs consumed 1 Kg of food per day or 15 mg/day. The female dogs consumed 800g of food or 12 mg/day. The average male terminal body weight was 15.2 kg, therefore the dose was 15 mg/15.2 kg/day or 0.98 mg/kg/day. The average female terminal body weight was 15.1, therefore the dose was 12 mg/15.1 kg/day or 0.79 mg/kg/day.

The amount of diet consumed may seem large, however the diet was made up of one part dry diet mixed with two parts water. Food consumption data was not listed in the report. The animals were given a fixed amount of food every day. The weight of any food left over the next day was recorded. Thus the dogs had 24 hours to eat their food ration. No data were presented in the report because all dogs ate all of their food every day. The laboratory could present data to show that the male dogs ate 1000g per day and the females ate 800g per day however the presentation would be superfluous. It is sufficient as stated in the report that no inhibition of food intake was observed.

EPA Conclusion 7:

A new multigeneration study must be submitted.

Response:

A new multigeneration study is included in this submission (Tab No. C-1). The survival effects in the F_2A and F_2B litters of the first study were not observed in the second study. It is concluded that the effects in the first study were due to a viral infection and not related to HOE 33171. Since the scientific question raised in the first study related only to pup weight and pup survival, the second study includes only an antemortem evaluation. No histopathology is included because it is not needed to resolve the scientific question.

EPA Conclusion 8

Consideration of need for repeating the mouse oncogenicity study.

Response:

The EPA review of the submitted data did not include the results after the one year interim sacrifice. The toxicologic profile in mice of HOE 33171 is illustrated in Table 5. At a dose of 5000 ppm for 32 days, HOE 33171 decreased the serum total lipids and increased alkaline phosphatase and liver weight. Histopathologic evaluation revealed hepatocellular hypertrophy and necrosis in the liver and kidney. Kidney necrosis was

observed at a dose as low as 315 ppm. At doses from 20 to 315 ppm HOE 33171 increased cholesterol, total lipids and liver weight. Hepatocellular hypertrophy was observed at doses as low as 20 ppm. Based on these findings the doses for the carcinogenicity study were set at 2.5, 10 and 40 ppm.

Since this study was designed to assess carcinogenicity only no evaluation of total lipids or cholesterol were performed. At the one year interim sacrifice there was an increase in the high dose liver and kidney weights with no evidence of hepatocellular hypertrophy. At the end of two years there was a statistically significant decrease in the female liver weight. Normally decreases in liver weight are not considered relevant, however this same pattern was observed in the rat chronic and oncogenicity studies (See Table 1). It is evident based on all of the studies that doses above 10 ppm produce effects in mice. As the studies progress, parallel to the rat studies, the mice adapt and the severity of the lesions diminish. Based on these results it is clear that HOE 33171 is not oncogenic and NOEL for the mouse study is 10 ppm. The effects observed at one year and in the earlier studies indicate that a maximum tolerated dose has been reached.

Teratology:

The EPA has concluded based on the results of a rabbit teratology study that HOE 33171 is teratogenic in rabbits.

Response:

The EPA's conclusion is based on the finding of diaphragmatic hernias in the 200 mg/kg dose group. This dose level of 200 mg/kg proved to be excessive for the evaluation of the teratogenic potential of HOE 33171. The dams lost 300 g during the treatment period. Food consumption was only 15% of the control value. Two out of 15 dams died during the study, an additional dam delivered prematurely and four more dams aborted. Pup weight, crown-rump length and placental weight were significantly reduced at the 200 mg/kg level. Of the pups that were born alive, only 61% survived 24 hours. It is clear from this data that the 200 mg/kg was excessive and that the information obtained from it cannot be used in the evaluation of the teratogenic potential of HOE 33171. The results of this study and a repeat study are shown in Table 6. It is clear from this data that a dose of 50 mg/kg is the maximum tolerated dose and that the NOEL is 10 mg/kg. HOE 33171 is not teratogenic at 10 or 50 mg/kg. At a dose of 200 mg/kg it is maternally and feto-lethal.

We have performed eight embryotoxicity studies with HOE 33171. These studies are summarized in Table 7. HOE 33171 was not teratogenic in any of the studies. In all cases the maternal NOEL was lower than the fetal NOEL. The results of these studies are consistent and clearly demonstrate that HOE 33171 is not teratogenic. Oral studies were performed in rats, rabbits and mice according to guidelines. These studies did not indicate any teratogenic potential. An additional study was done in monkeys. The objective of this study was to determine if maternally toxic doses have any potential for producing adverse effects on the fetus. This study was not

intended to demonstrate a NOEL. The results of this study demonstrate that at maternally toxic or lethal doses HOE 33171 does not affect the fetus.

A rat post-natal type study was performed to determine if exposure during gestation had delayed effects on fetal development after birth. The dose levels and dose timing were the same as those in the rat oral teratology study. The results of this study are included in this submission (Tab No C-2). This study demonstrates that no effects appear during postnatal development as a result of exposure during organogenesis.

Dermal teratology studies were performed in rats and rabbits to evaluate potential adverse effects after dermal exposure. These studies did not indicate any teratogenic response. The EPA review of the studies concluded that there were no effects, however the studies were deficient since no information on HOE 33171 dermal absorption was presented. Included in this submission are the results of a rat dermal absorption study according to the proposed guidelines (Tab No C-10). This study indicates that HOE 33171 is absorbed. Approximately 77% of the test material was bound to the skin and absorbed. Even after the material was washed from the skin, the material bound to the skin continued to be absorbed. These results are supported by the four week dermal toxicity study (Accession No. 255851) in which the NOEL was 20 mg/kg. Doses of 100 and 500 mg/kg clearly produced effects on the animals.

The results of the dermal penetration and toxicity studies show that HOE 33171 is absorbed. The dermal teratology studies demonstrate that even though HOE 33171 is absorbed there is no potential for adverse effects on

the fetus. We feel that the results of the reproduction, post[illegible] teratology studies prove that there is no risk to the fetus after [illegible] exposure. HOE 33171 is not teratogenic. None of the studies give an indication of an adverse effect on the fetus. This position is summarized in a document entitled "Summary and Evaluation of the Embryotoxicity Data of HOE 033171" (EPA Accession No. 256666).

HOE 33171 - Summary of Toxicology Profile in Rats

RATION	DOSE (PPM)	TOTAL LIPIDS	CHOLESTEROL	ALKALINE PHOSPHATASE	LIVER WEIGHT	KIDNEY WEIGHT	HISTOPATHOLOGY LIVER	HISTOPATHOLOGY KIDNEY	ACCUMULATION
Days	2	ND	ND	ND	NC	NC	NC	NC	
	20	ND	ND	ND	NC	NC	NC	NC	
DAYS	80	NC	Decrease	NC	NC	NC	NC	NC	
	315	Decrease	Decrease	NC	Increase	Increase (M)	NC	NC	
	1250	Decrease	Decrease	Increase	Increase	Increase	NC,M	NC	
	5000	Decrease	Decrease	Increase	Increase	Increase	NC,M	NC	
Days Wks)	20	NC	NC	NC	NC	ND	NC	ND	
	80	NC	Decrease (M)	NC	NC	ND	ND	ND	
	320		Decrease	NC	ND	ND	ND	ND	
3 Wks)	20	NC	NC	NC	NC	NC	NC	NC	
	80	Decrease	Decrease (M)	NC	NC	NC	NC	NC	
	320	Decrease	Decrease	Increase	Increase	Increase	NC	NC	
rcinogenicity 6 Wks)	5	Decrease (T)	NC	NC	ND	ND			
	30	Decrease (T)	Decrease	NC	ND	ND			
	180	Decrease (T)	Decrease	Increase (M)	ND				
4 Wks)	5	NC	NC	NC	Decrease (T)	NC		N	
	30	NC	Decrease (F)	NC	Decrease (T)	NC		NA	
	180	Decrease	Decrease	NC	Decrease (T)	Increa			
9 Wks)	5	ND	NC	NC	ND	ND			
	30	ND	NC	NC	ND	ND			
	180	ND	NC	NC	ND	ND			
06 Wks)	5	NC	NC	NC	NC	NC			
	30	NC	NC	NC	Decrease	NC			
	180	Decrease	Decrease	Increase(T:M)	Decrease	NC			
20 Wks)	5	ND	ND	ND	NC	NC	NC		
	30	ND	ND	Decrease	NC	NC	NC		
	180	ND	ND	ND	Decrease	NC	NC		

TABLE 2

Comparison of Oral and Inhalation Toxicity of HOE 33171 in Rats

URATION	DOSE	TOTAL LIPIDS	CHOLESTEROL	ALKALINE PHOSPHATASE	LIVER WEIGHT	KIDNEY WEIGHT	HISTOPATHOLOGY LIVER	HISTOPATHOLOGY KIDNEY	ACCESSION NO.
Week Inhal.	15 (mg/m^3)	NC	NC	NC	NC	NC	ND	ND	255850
	73	Decrease	Decrease (M)	Increase (M)	Increase	Increase	NC	NC	
	248	Decrease	Decrease	Increase	Increase	Increase	HM	NC	
	727	Decrease	Decrease	Increase	Increase	Increase	HM	NC	
Week recovery	15 (mg/m^3)	NC	NC	NC	ND	ND	ND	ND	255850
	73	NC	NC	NC	ND	ND	ND	ND	
	248	NC	NC	NC	NC	NC	ND	ND	
	727	NC	NC	NC	NC	NC	ND	ND	
3 Week Oral	20 (ppm)	NC	NC	NC	NC	NC	NC	NC	071789
	80	Decrease	Decrease (M)	NC	NC	NC	NC	NC	
	320	Decrease	Decrease	Increase	Increase	Increase	HM	NC	
3 Week with Week recovery	20 (ppm)	NC	NC	NC	NC	NC	NC	NC	071789
	80	Decrease	NC	NC	NC	NC	NC	NC	
	320	Decrease	NC	NC	NC	NC	NC	NC	

NC = No Change ND = Not Determined T = Trend (M) = Males only (F) = Females only HM - Hepatomegaly N = Necrosis

TABLE 3

Comparison of Oral and Dermal Toxicity of HOE 33171

DURATION	DOSE	TOTAL LIPIDS	CHOLESTEROL	ALKALINE PHOSPHATASE	LIVER WEIGHT	KIDNEY WEIGHT	HISTOPATHOLOGY LIVER	HISTOPATHOLOGY KIDNEY	ACCESSION NO.
4 Week Dermal	20 (mg/kg)	Decrease (M)	Decrease (M)	NC	NC	NC	NC	NC	255851
	100	Decrease (M)	Decrease (M)	NC	Increase	NC	NC	NC	
	500	Decrease (M)	Decrease (M)	Increase	Increase	NC	NC	NC	
4 Week Recovery	20 (mg/kg	NC	NC	NC	ND	ND	NC	NC	255851
	100	NC	NC	NC	NC	NC	NC	NC	
	500	NC	NC	NC	NC	NC	NC	NC	
13 Week Oral	20 (ppm)	NC	NC	NC	NC	NC	NC	NC	071789
	80	Decrease	Decrease (M)	NC	NC	NC	NC	NC	
	320	Decrease	Decrease	Increase	Increase	Increase	HM	NC	
13 Week with 4 Week recovery	20 (ppm)	NC	NC	NC	NC	NC	NC	NC	071789
	80	Decrease	NC	NC	NC	NC	NC	NC	
	320	Decrease	NC	NC	NC	NC	NC	NC	

NC = No Change ND = Not Determined T = Trend (M) = Males only (F) = Females only HM = Hepatomegaly N = Necrosis

101

005632

TABLE 4

HOE 33171

2 YEAR DOG STUDY (MALES)

Dose (ppm)	BODY WEIGHT		
	Initial	Final	Change
0	13.6 ± 0.5	17.1 ± 1.0	3.5 ± 1.1
3	13.6 ± 1.3	16.5 ± 1.7	2.9 ± 0.9
15	12.5 ± 1.0	*15.2 ± 0.8	2.7 ± 0.8
75	13.5 ± 0.6	*15.5 ± 0.6	*1.9 ± 0.6

Significant Change Only On BW Change at 75 ppm.

Change In Terminal BW Due To Initial Body Weight Differences.

Therefore NOEL = 15 ppm.

TABLE 5

Summary of Toxicology Profile in Mice

DURATION	DOSE (PPM)	TOTAL LIPIDS	CHOLESTEROL	ALKALINE PHOSPHATASE	LIVER WEIGHT	KIDNEY WEIGHT	HISTOPATHOLOGY LIVER	HISTOPATHOLOGY KIDNEY	ACCESSION NO.
28 Days	2	~~ND~~	~~ND~~	~~ND~~	NC	NC	NC	NC	This submission
	20	NC	NC	NC	NC	NC	Catalase Increase	NC	
30 Days	5	NC	NC	NC	NC	NC	NC	NC	071791
	10	NC	NC	NC	NC	NC	NC	NC	
	20	Increase (M)	Increase	NC	Increase (F)	NC (pu)	HM	NC	
	80	Increase (M)	Increase	NC	Increase	NC (pu)	HM	NC	
32 Days	80	Increase	Increase	NC	Increase	NC	HM	NC	071790
	315	Increase	Increase	Increase	Increase	Increase	HM	N (F)	
	1250	NC	NC	Increase	Increase	Incresase	HM	N (F)	
	5000	Decrease (M)	~~ND~~	Increase	Increase	Decrease	HM,N	N (F)	
1 Year	2.5	~~ND~~	~~ND~~	NC	NC	NC	NC	NC	255855
	10	~~ND~~	~~ND~~	NC	NC	NC	NC	NC	
	40	~~ND~~	~~ND~~	NC	Increase (M)	Increase	NC	NC	
2 Year	2.5	~~ND~~	~~ND~~	NC	NC	NC	NC	NC	258955
	10	~~ND~~	~~ND~~	NC	NC	NC	NC	NC	
	40	~~ND~~	~~ND~~	NC	Decrease (F)	NC	NC	NC	

NC = No Change (M) = Male only PU = Proteinuria (F) = Female only HM = Hepatomegaly N = Necrosis

TABLE 6

Summary of Rabbit Embryotoxicity Studies

DOSE (mg/kg)	WEIGHT GAIN (g)	FOOD CONSUMPTION (% of control)	DEATH (%)	ABORTIONS (%)	FETAL TOXICITY	DIAPHRAGMATIC HERNIA	ACCESSION NO.
12.5	NC	NC	NC	NC	NC	NC	256667
50	-12	69	NC	13	TREND	NC	
200	-297	15	13	31	MARK.INC.	+	
2	NC	NC	NC	NC	NC	NC	256667
10	NC	NC	NC	NC	NC	NC	
50	NC	87	NC	NC	NC	NC	

NC = No Change + = Present

TABLE 7

SUMMARY OF HOE 33171 EMBRYOTOXICITY STUDIES

Species	Route	NOEL (mg/kg body weight/day) Dams	Fetuses
Mouse	Oral	10	> 50
Rat	Oral	10	> 10
Rat	Dermal	> 1000	> 1000
Rabbit	Oral	10	> 10
Rabbit	Dermal	100	> 1000
Monkey	Oral	< 10	> 50

005632

DRAFT POSITION PAPER ON

THE MAXIMUM TOLERATED DOSE (MTD)

To assist in the selection of doses to be used in oncogenicity testing and in the evaluation of such studies by the Toxicology Branch of the Office of Pesticide Programs at the EPA, a position paper has been drafted to define the concept of the maximum tolerated dose (MDT), how to predict it, and how to determine the need for repeating completed studies without MTD levels.

As part of the peer-review process, the Agency requests that the Science Advisory Panel comment on our interpretation of the MTD concept in oncogenicity testing.

Date: April 10, 1986

- TOXICOLOGY BRANCH -

POSITION PAPER ON MAXIMUM TOLERATED DOSE (MTD) IN ONCOGENICITY STUDIES

Contributors: Jane E. Harris, Ph.D.
Theodore M. Farber, Ph.D.
Reto Engler, Ph.D.
John A. Quest, Ph.D.
Clint S. Skinner, Ph.D.

April 1986

Introduction

Many guidelines for oncogenicity studies in laboratory animals, including those from both domestic and international regulatory agencies, specify the use of a maximum tolerated dose (MTD) (1-16). The inclusion of this concept in long-term bioassays is primarily to maximize the detection of a potential oncogenic response without significantly altering the accuracy and interpretability of the data obtained by simultaneously eliciting excessive toxicological effects, including mortality or overwhelming normal metabolic processes (16).

There are several common toxicological criteria included in definitions of the MTD in guidelines of oncogenicity studies. Foremost among these is that the highest dose level tested should not reduce survival except by tumor-related mechanisms. In addition, definitions agree that this dose level should evoke some minimally sufficient toxicity. It is often and practically speaking stated that the MTD is a predicted value and as such should be estimated on the basis of toxicity data obtained in subchronic (i.e., 90-day) or range-finding studies (7,13,17).

Several parameters have been considered in predicting a dose based on subchronic toxicity. For example, it is generally agreed that body weight gain, the primary toxicity considered in almost every guideline, should not be reduced by more than 10% by the highest dose level (1). Other guidelines indicate that the MTD should be based on clinical signs of toxicity and pathologic lesions. In addition, other recommendations are that a MTD should be determined for each strain of animal (7), that it should not exceed 5% of the total diet, (i.e., 50,000, ppm) in dietary studies (16), and that it should be determined based upon metabolic considerations related to saturation kinetics (17,18).

Despite general agreement among scientists regarding toxicological parameters that should be used to estimate the MTD level, there nevertheless appears to be a lack of consensus regarding the definition of an MTD (1,17). That is, guidelines often vary with respect to precise definitions of toxic endpoints to determine the MTD, or the degrees of toxicity required to confirm that the dose was the MTD. In fact, the term MTD has been described by almost as many different subjective terms as there are individuals who use it (16). This has frequently led to confusion and differences in the interpretation of results between regulatory agencies and industrial firms at the termination of animal oncogenicity studies. This problem has not gone unnoticed; several efforts, primarily via the National Toxicology Program (NTP), are being expended to more clearly describe the MTD concept (17,19) and bring consistency to the definition.

Within the various Environmental Protection Agency (EPA) guidelines for oncogenicity studies, there are apparent differences in the definition of the MTD. For example, the earlier 1978 EPA guidelines provided extensive definitions (16) whereas the more recent 1982 guidelines do not (14,15). In addition, some inconsistencies can be found regarding the MTD definitions within the Agency itself. Because of this, and in an effort to improve scientific and regulatory decisions on oncogenicity studies within the Office of Pesticide Programs, the present document has been developed by the Toxicology Branch, Hazard Evaluation Division, Office of Pesticide Programs.

This document has been developed for the following three reasons:

1. To describe how toxicity data obtained from subchronic studies can be used to estimate the MTD level in oncogenicity studies.

2. To define the MTD level in oncogenicity studies, and to describe the types of toxicity that satisfy requirements for assessing the MTD in oncogenicity studies.

3. To describe a decision tier scheme to determine the need to repeat completed oncogenicity studies in which MTD levels were not reached.

1. Use of Toxicity Data from Subchronic Studies to Estimate the MTD Level in Oncogenicity Studies

The Toxicology Branch believes that in evaluating the acceptability of chronic/oncogenicity studies, consideration should be given to the concept that the MTD is a predicted value derived from observed toxicities in subchronic or range-finding studies. The MTD should be selected near a level which resulted in toxicity in the subchronic or range-finding study. Based on this principle if the highest dose was predicted from observed toxicities in subchronic studies, but adaptation occurred during chronic exposure to negate these toxic effects, then the chronic study would still meet our scientific standards and, thereby, would not need to be repeated because of an absence of a MTD.

To the contrary, a chronic/oncogenicity study without a demonstrated effects would be considered unacceptable (supplementary) as an oncogenicity test if the highest dose was not predicted from observed toxic effects in the subchronic study.

Several measured toxicological parameters in subchronic or range-finding studies are important for predicting the MTD level in oncogenicity studies.

- The most commonly observed toxicity used in selecting a MTD is a statistically significant decrement in body weight gain. The Toxicology Branch believes this decrement in body weight gain should ideally reach about 10% in subchronic testing.
- Consideration of other observed toxicities, (i.e., nonneoplastic pathology data, serum enzyme changes, and clinical signs) in predicting a MTD may also be justified if these toxicities may be expected upon chronic exposure to result in shortening the lifespan of the animal, thereby decreasing the time on test. For example severe histopathological lesions in target organs, such as the liver, kidney, heart, etc., or marked depressions in hematological values suggesting leucopenia or anemia, or inhibition of cholinesterase to clinically toxic levels, may all result in reduced survival upon prolonged compound-exposure. Therefore, such demonstrated short-term toxicities should be considered when predicting the MTD for the chronic/oncogenicity study. On the other hand, minor treatment-related changes observed at the highest dose level of the subchronic test do not necessarily qualify this level as a

valid predictor of the MTD, e.g., blood cholinesterase inhibition without significant clinical signs, and liver weight increases or cellular hypertrophy suggestive of microsomal enzyme induction.

Several other important factors require consideration in dose selection in oncogenicity studies:

- A properly conducted oncogenicity study should include at least two doses lower than the MTD not only to help validate responses observed at the MTD level and to develop dose-response curve information, but also to provide a margin of safety against overestimating the MTD. Thus, in a three dose study, the mid-dose should be set at one-half the high dose; in the event that survival was too low at the highest dose because of severe toxicity, the mid-dose would then become the MTD used to evaluate potential oncogenicity.

- An MTD level should be determined for each species and strain to be used in an oncogenicity study.

- Pharmacokinetic and metabolism data for the test chemical, if available, should be utilized in the dose selection process. For example, significant observed differences in the pharmacokinetic or metabolic profile of the test substance between the high dose and lower doses should suggest the need for the inclusion of another dose in the study. This optional dose should approximate the maximal dose which yields pharmacokinetics similar to the lower doses. Pharmacokinetic and metabolism data should also be used in an evaluation of the biological significance of any oncogenic effects that may be observed.

It is strongly advised that the sponsor present, in writing, evidence regarding the MTD level they wish to utilize in an oncogenicity study or meet with toxicologists in OPP to discuss what they believe is the MTD level for chronic bioassays before the initiation of such studies, in order to avoid the use of inadequate levels of dosing in these studies.

2. Definition of the MTD Level in Oncogenicity Studies and Description of Types of Toxicity that Satisfy Requirements for Assessing the MTD

The Toxicology Branch of the Office of Pesticide Programs frequently encounters oncogenicity and chronic feeding studies in which the dose levels selected for chemical evaluation were not sufficiently great to evoke acceptable minimal signs of toxicity in the test animals. The failure to observe toxicity in an oncogenicity study at the highest dose level tested may tend to compromise interpretation of the oncogenic potential of the compound under study. That is, the test animal may not have been sufficiently challenged under the condition of the study to serve as a model for extrapolation of risk to humans.

Although the importance of attaining a MTD level in an oncogenicity study is commonly stressed, a consensus in the definition of the MTD has not been attained (see Introduction Section) and toxicologic variables to satisfy the requirement for assessing the MTD level have not been generally described. This has often inadvertently resulted in oncogenic studies where the highest dose level has produced little or no toxicologic effects. Therefore, the Toxicology Branch would like to define what is a MTD level for oncogenicity studies and also describe types of toxicity that should be observed in these studies. The MTD level is defined as a predictive dose obtained from analysis of subchronic study doses. In the oncogenicity study, the MTD should elicit toxicity without substantially altering the normal life span of the test species from effects other than tumor formation. Toxicologic parameters under investigation may include the following: decrements in body weight gain, hematologic effects, histopathology, or clinical chemistry, urinalysis, organ weight changes and neurological symptoms. Attempts should be made when possible to correlate these effects with clinical signs of toxicity or histopathologic changes in tissues or organs showing a toxic response to the chemical under test. Examples are given below of types of toxicity observed in an oncogenicity studies which demonstrate that the MTD or the highest dose tested (HDT) sufficiently expressed the full range of toxicity of the test substance.

- Body weight effects: Significant decrement in cumulative body weight gain approaching 10% in the first 90 days.

- Histopathology: Changes observed microscopically in tissues suggesting neoplastic events or significant cellular alterations, such as vacuolation and fatty changes in tissues.

- Hematologic effects: Significant alterations involving microcyctic or hemolytic anemia, changes in the prothrombin clotting time, leucopenia or leukemia or significant alteration in the relative number of other critical blood elements.

- Clinical chemistries: Depression of at least two of the three assayed cholinesterase enzyme measurements, i.e., plasma, red blood cell or brain acetylcholinesterase levels. Alternatively, significant alterations in clinical chemistry parameters such as: asparate aminotransferase (SGOT), alanine aminothransferase (SGPT), alkaline phosphatase, proteins, cholesterol, blood urea nitrogen, glucose, etc. which can be correlated with histopathological alterations or biochemical changes in corresponding organs.

- Urinalysis: Significant alterations in urinalysis parameters which are correlated with histopathological alterations or biochemical changes in corresponding organs.

- Organ weight changes: Significant decreases or increases in relative organ to body or brain weight ratios which can be correlated with histopathological alterations in the corresponding organ. (The MTD is not considered reached if increases in relative liver to body weight ratios occur in the absence of significant cellular alterations in the liver; these increases are more likely to result from microsomal enzyme induction, representing an adaptive or pharmacologic response of the liver to the test chemical rather than a toxic response.)

It should be noted that if survival is compromised at the high dose in an oncogenicity study because of extensive compound-related toxicity,the mid dose level (which should be approximately one-half the high dose level in magnitude) may then satisfy the MTD level for the study.

In oncogenic studies of pesticides, a dose of 1 gram/kg body weight/day should provide an adequate upper limit for extrapolating animal data to humans. This dose of 1 gm/kg body wt/day is equivalent to dietary concentrations of approximately 20,000 ppm (i.e., 2%) in the rat and 7,000 ppm (i.e., 0.7%) in the mouse. The dose level of 1 gram/kg body weight/day is considered as an adequate uppermost level for testing because pesticides are relatively potent agents with respect to the induction of toxic effects in animals as compared to some other chemicals. For example, an uppermost exposure level of 5% is employed by the FDA for food additives, but these are generally of lower toxicological potency than most pesticides.

3. Decision Tier Scheme to Determine the Need to Repeat Completed Oncogenicity Studies Without MTD Levels

The Toxicology Branch believes that a consistent practice of using information derived from subchronic toxicity studies to estimate a MTD level for newly initiated oncogenicity studies will improve the usefulness of the data obtained as well as subsequent regulatory decision processes. At the same time, the Toxicology Branch is also aware that oncogenicity studies in progress or already completed are subject to scientific review and re-review by the EPA using the principles described in this document. Under such circumstances, occasional oncogenicity studies may be determined not to have been performed using a MTD level, thereby raising concerns over their adequacy and whether or not they should be retested. In order to evaluate the need for retesting of these studies, the Toxicology Branch proposes the use of a decision tier scheme in which additional relevant toxicity data on a chemical will also be considered to evaluate its oncogenic potential. The decision tier scheme contains the following criteria levels that will be used to determine the need for retesting of oncogenicity studies which showed no oncogenicity or minimal toxicity and were begun (in life portion) before (date of FR rule).

LEVEL 1 - Nearness to the Apparent MTD

If the highest dose tested (HDT) is greater than or equal to one half the apparent MTD, as judged from subchronic data or other chronic studies, no retesting is required.

If, however, the HDT is less than one half the apparent MTD, consideration at the next decision level (level 2) is required.

LEVEL 2 - Demonstrated Oncogenicity

If the test substance is demonstrated to be an oncogen in another species, retesting is required.

If, however, the test substance did not demonstrate oncogenic potential in an acceptable study in another species, consideration at the next level (level 3) is required.

LEVEL 3 - Genotoxicity

If a clear demonstration of genotoxicity in the test substance or major metabolite in a battery of acceptable mutagenicity studies designed to detect gene, chromosome, or DNA effects is attained, retesting is required.

Peer review by two or more qualified experts is necessary to determine positive genotoxic potential.

If no genotoxicity is demonstrated in an acceptable battery of tests including one study each to detect effects at the gene, chromosome, and DNA level, consideration at the next level (level 4) is required.

LEVEL 4 - Oncogenicity of Structural Analogs

If structural analogs of the test substance or known metabolites have been shown to be oncogenic in animals or man, retesting is required.

If structural analogs of the test substance have not been shown to be oncogenic, consideration at the next highest level (level 5) is required.

LEVEL 5 - Absolute Value of Highest Dose Tested

If the highest exposure tested is 0.5 Gm/kg b.w./day, no retesting is required.

If, however, the highest exposure tested is significantly less than 0.5 Gm/kg b.w./day, consideration at the next level (level 6) is required.

LEVEL 6 - Highest Dose Tested Relative to Dose tested in Second Species of an oncogenicity study with an MTD

If the highest dose tested in the study under evaluation expressed in mg/kg/day, is at least equal to the HDT in mg/kg/day in an acceptable oncogenicity study in another species, then no retesting is required.

If, however, the highest dose tested in the study, expressed in mg/kg/day, is less than the HDT in mg/kg/day in an acceptable study in another species, consideration at the next level (level 7) is required.

LEVEL 7 - Margin of Safety Calculated for Highest Dose Tested vs Human Exposure

If the margin of safety (ratio) between the highest dose tested and the highest expected level of human exposure is greater than or equal to 1000, no retesting is required.

If the margin of safety (ratio) between the highest dose tested and the highest expected level of exposure is between 100 and 1000, the need for retesting is discretionary.

If, however, the margin of safety between the highest dose tested and the highest expected level of exposure is less than 100, retesting is required.

REFERENCES

1. International Life Sciences Institute (ILSI)(1984). The selection of doses in chronic toxicity/carcinogenicity studies. In: Current Issues of Toxicology, (H.C. Grice, ed.), pp. 9-49, Springer-Berlag, New York.

2. Clayson, D.B.(1962). Testing chemicals for carcinogenic activity. I.Methods. In: Chemical Carcinogenesis, pp. 55-85, J. and A: Churchill, Ltd., London.

3. Weisburger, J.H. and Weisburger, E.K. (1967). Tests for chemical carcinogenesis. In: Methods in Cancer Research (H. Busch, ed.) Vol. 1;pp 307-398, Academic Press, New York.

4. WHO (1969). Principles for the testing and evaluation for carcinogenicity. Report of a WHO scientific group. World Health Organization Technical Report, Series No. 426. Geneva.

5. Friedman, I. (1974). Section 4. Report of Discussion Group No. 3. Dose selection and administration. In: Carcinogenesis Testing of Chemicals (L. Golberg, ed.) pp. 21-22, CRC Press, Inc., Cleveland.

6. Health and Welfare Canada (1975). The Testing of Chemicals for Carcinogenicity, Mutagenicity, and Teratogenicity. pp. 2-31, Health and Welfare Canada, Ottawa.

7. Sontag, J.M., Page, N.P., and Saffiotti, U. (1976). Guidelines for Carciogen Bioassay in Small Rodents. DHHS Publication (NIH) 76-801 National Cancer Institute, Bethesda, MD

8. WHO (1978). Principles and methods for evaluating toxicity chemicals, Part 1. Environmental Health Criteria, Vol. 6, World Health Organization, Geneva.

9. IRLG: Interagency Regulatory Liaison Group (1979). Work Group on Risk Assessment. Scientific bases for identification of potential. J.Nat. Cancer Inst. 63, 241-268.

10. CEC: Commission of the European Communities (1980). Food-science and techniques. Reports of the scientific committee for food (tenth series). Guidelines for the Safety Assessment of Food Additives.

11. IARC: International Agency for Research on Cancer (1980). Long-term and Short-term Screening Assays for Carcinogenesis: A Critical Appraisal Supplement 2, IARC, Lyon.

12. OECD: Organization of Economic and Cooperative Development (1981). OECD Long-term Expert Group: Test Guidelines for Carcinogenicity Studies.

13. U.S. Environmental Protection Agency (1978), Pesticide Programs Guidelines for Registering Pesticides in the U.S.; Hazard Evaluation: Humans and Domestic Animals.

14. U.S. Environmental Protection Agency (1982), Pesticide Assessment Guidelines, Subdivision F, Hazard Evaluation: Human and Domestic Animals.

15. U.S. Environmental Protection Agency (1982), Health Effects Test Guidelines, Office of Toxic Substances.

16. Paynter, O.E. (1985). Hazard Evaluation Division, Standard Evaluation Procedure. Oncogenicity Potential (Guidance for Analysis and Evaluation of Long-Term Rodent Studies). U.S. Environmental Protection Agency. pp. 1-109.

17. Haseman, J.K. (1985). Issues in Carcinogenicity Testing: Dose Selection. Fund. Appl. Toxicol. 5: 66-78.

18. Weiner, M. and Newberne, J.W. (1977). Drug Metabolites in the Toxicologic Evaluation of Drug Safety. Toxicol. Appl. Pharmacol. 41: 231-233.

19. Doull, J., (Chairman) (1984). Ad Hoc Panel on Chemical Carcinogenesis Testing and Evaluation. Draft report. 2/15/84, pp. 129-157. NTP Board of Scientific Counselors.