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

005709

FEB 3 1987

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Review of 2 acute inhalation studies and 2 mutagenicity studies with Metribuzin (SENCOR®) submitted as part of data call-in for the Metribuzin Registration Standard. Tox Branch Project No's. 1773, 1774, 1775 & 1776, Caswell No. 33D.

TO: Robert J. Taylor/Vickie Walters, PM #25
Herbicide-Fungicide Branch
Registration Division (TS-767C)

FROM: Stephen C. Dapson, Ph.D. Stephen C. Dapson 1/30/87
Pharmacologist, Review Section V
Toxicology Branch/HED (TS-769C)

THRU: Quang Q. Bui, Ph.D., D.A.B.T. Quang Bui 2/3/87
Acting Section Head, Review Section V
Toxicology Branch/HED (TS-769C)
and
Theodore M. Farber, Ph.D., D.A.B.T. 2/3/87
Chief, Toxicology Branch
Hazard Evaluation Division (TS-769C)

ACTION REQUESTED: Review of 2 acute inhalation studies and 2 mutagenicity studies with Metribuzin (SENCOR®), submitted as part of data call-in for the Metribuzin Registration Standard.

Recommendations:

The registrant has fufilled the data call-in requirements for the Metribuzin Registration Standard relative to the following Guideline required studies.

For Generic Data Requirements Table A:

§81-3 (Acute Inhalation Toxicity - Rat) with EPA Record # 171478 (Mobay Ag Chem # 91754).

§84-1 (Gene Mutation [Ames Test]) with EPA Record #'s 171481 and 171482 (Mobay Ag Chem #'s 91759 & 91760)

For Generic Data Requirements Table B:

§81-3 (Acute Inhalation Study - Rat, Technical and Formulation) with EPA Record #'s 171478 & 171479 (Mobay Ag Chem # 91754 & 91758).

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Conclusions relative to previously stated studies:

For the acute inhalation study with Metribuzin (SENCOR®), EPA Record # 171478 (Study # 85-041-18, Mobay Ag Chem # 91754), the LC_{50} is greater than the gravimetric concentration of 648 mg/m^3 [0.648 mg/l] for male and female rats. This dose is considered the maximum obtainable concentration for the equipment used and it produces no compound related mortality. Core Classification: Core-Minimum Data, although only one dose was used, it can be considered the maximum obtainable concentration. Toxicity Category: Tox Cat II.

For the acute inhalation study with SENCOR® 50% Wettable Powder, EPA Record # 171479 (Study No. 85-041-01, Mobay Ag Chem # 91758), the LC_{50} for SENCOR® 50% Wettable Powder is greater than the gravimetric concentration of 2123 mg/m^3 [2.12 mg/l] for male and female rats. This dose is considered the maximum obtainable concentration for the equipment used and it produces no compound related mortality. Core Classification: Core-Minimum Data, although only one dose was used, it can be considered the maximum obtainable concentration. Toxicity Category: Tox Cat III

For the mutagenicity test, Unscheduled DNA Synthesis in Rat Primary Hepatocytes, EPA Record # 171481 (MA Study No. T4485.380, Mobay Ag Chem # 91759), under the conditions of this test. Metribuzin did not cause a significant increase in the Unscheduled DNA Synthesis (UDS) as measured in this study, whereas the positive control did increase UDS. Core Classification: Acceptable.

For the mutagenicity test, CHO/HGPRT Mutation Assay in the Presence and Absence of Exogenous Metabolic Activation, EPA Record # 171482 (MA Study No. T4485.332, Mobay Ag Chem No. 91760), under conditions of this study, Metribuzin is negative in the CHO/HGPRT mutation assay. Core Classification: Acceptable.

DLR 1

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I. Study Type: Acute Inhalation Toxicity
(Guideline 81-3)

Study Title: Acute Inhalation Toxicity Study with Metribuzin (SENCOR®) in Rats

EPA Identification Numbers: EPA ID No. 3125-270
EPA Accession No. 262227
EPA Record No. 171478
Shaughnessy Code: 101101-4
Caswell No. 33D
Tox Branch Project No. 1773
Document No.

Sponsor: Mobay Chemical Corporation

Testing Laboratory: Mobay Chemical Corporation
Environmental Health Research
Corporate Toxicity Department
17745 Metcalf
Stilwell, Kansas 66085

Study Numbers: Study Number 85-041-18
Toxicology Report No. 727
Mobay Ag Chem # 91754

Study Date: March 17, 1986

Study Author: R. N. Shiotsuka

Test Material: SENCOR (also known as Metribuzin)
4-amino-6-(1,1-dimethylethyl)-3-(methylthio)
-1,2,4-triazin-5(4H)-one
92.6% a.i. (determined within one month of the study)
Reference No. 77-297-50
Stability - 2 years shelf life

Vehicle: No vehicle used

Test Animal: Rat, Sprague-Dawley (Rattus norvegicus), males and females
weight = 189 to 243 gm for males
183 to 206 gm for females
obtained from Sasco, Inc., Omaha NE.

II. Reviewed by: Stephen C. Dapson, Ph.D. Stephen C. Dapson 1/30/87
Review Section V, Toxicology Branch/HED
Secondary Review by: Quang Q. Bui, Ph.D., D.A.B.T.
Acting Section Head, R.S. V/T.B./HED Bui 2/3/87

This study was designed to evaluate the acute inhalation toxicity of metribuzin generated as a dust to male and female rats.

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III. Materials and Methods: A copy of the "Methods" section from the investigators report is appended. The following comments and highlights are noted:

Only 1 (one) exposure concentration was used, 648 mg/m^3 air [0.648 mg/l]. According to the investigators this was the maximum obtainable concentration with the equipment used. The equipment used for the generation of the "dust", the animal exposure procedure, the treatment of exhaust, the measurement of particle size distribution, the nominal concentration and gravimetric concentration calculations are described in the attached "Methods" section.

Twenty (20) animals per sex were used, the weight but not the age of the rats was provided.

The animals were exposed once for 4 hours. They were observed for mortality and signs of toxicity during exposure and at "approximately" 0.5, 1.0 and 2.5 to 3.0 hours after exposure. The animals were then observed twice at day for 14 days following exposure to the test material.

Individual body weights were taken prior to exposure and on days 3, 7 and 14 days following exposure.

All surviving animals were sacrificed at 14 days and a complete post-mortem examination was conducted on each rat.

IV. Results:

No deaths were reported from the 4 hour exposure to the test compound in this study.

According to the investigators, the only compound related sign of toxicity was transient salivation observed in both males and females. The exposure technique used apparently caused ocular and nasal irritation and lacrimation. This was observed in both control and treated animals. One control rat suffered an eye injury and other animals showed neck edema which was apparently related to the exposure procedure.

No effect was noted on body weight or body weight gain (both individual animal and mean data were provided).

Post-mortem examinations conducted at 14 days showed no compound related effects. One treated female has a small, pitted kidney and 1 control female had a white eye zone.

The gravimetric mean was determined to be 648 mg/m^3 [0.648 mg/l] (nominal concentration was 20,547 mg/m^3 [20.55 mg/l]). The mean particle size (mass median aerodynamic diameter) was found to be 5.1 um with a geometric standard deviation of 2.1 um.

Temperature range during exposure was 23.0 to 25.7°C for control and 23.4 to 25.4°C for treated animals. Relative humidity range was 15 to 34% for control and 13 to 62% for treated animals.

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The LC_{50} is greater than the gravimetric concentration of 648 mg/m^3 [0.648 mg/l] for male and female rats.

This dose is considered the maximum obtainable concentration for the equipment used and it produces no compound related mortality.

V. Core Classification: Core-Minimum Data, although only one dose was used, it can be considered the maximum obtainable concentration.

Toxicity Category: Tox Cat II

METRIBUZIN

RIN: 3187-91

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Pages 6 through 9 are not included.

The material not included contains the following type of information:

____ Identity of product inert ingredients.

____ Identity of product impurities.

____ Description of the product manufacturing process.

____ Description of quality control procedures.

____ Identity of the source of product ingredients.

____ Sales or other commercial/financial information.

____ A draft product label.

____ The product confidential statement of formula.

____ Information about a pending registration action.

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DER II

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1. Study Type: Acute Inhalation Toxicity
(Guideline §81-3)

Study Title: Acute Inhalation Toxicity Study with SENCOR® 50% Wettable Powder Dust in Rats

EPA Identification Numbers: EPA ID No. 3125-270
EPA Accession No. 262227
EPA Record No. 171479
Shaughnessy Code: 101101-4
Caswell No. 33D
Tox Branch Project No. 1774
Document No.

Sponsor: Mobay Chemical Corporation

Testing Laboratory: Mobay Chemical Corporation
Environmental Health Research
Corporate Toxicity Department
17745 Metcalf
Stilwell, Kansas 66085

Study Numbers: Study Number 85-041-01
Toxicology Report No. 731
Mobay Ag Chem # 91758

Study Date: March 27, 1986

Study Author: R. N. Shiotsuka

Test Material: SENCOR 50% Wettable Powder
51.5% a.i. of SENCOR Technical, Grade 2 or Grade 1
(also known as Metribuzin)
4-amino-6-(1,1-dimethylethyl)-3-(methylthio)
-1,2,4-triazin-5(4H)-one
Stability - no chemical deterioration for at least
4 months at 40°C.

Vehicle: No vehicle used

Test Animal: Rat, Sprague-Dawley (Rattus norvegicus), males and females
weight = 189 to 271 gm for males
182 to 215 gm for females
obtained from Sasco, Inc., Omaha, NE.

II. Reviewed by: Stephen C. Dapson, Ph.D. Stephen C. Dapson 1/30/87
Review Section V, Toxicology Branch/HED
Secondary Review by: Quang Q. Bui, Ph.D., D.A.B.T.
Acting Section Head, R.S. V/T.B./HED Bui 2/3/87

This study was designed to evaluate the acute inhalation toxicity of SENCOR 50% Wettable Powder generated as a dust to

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III. Materials and Methods: A copy of the "Methods" section from the investigators report is appended. The following comments and highlights are noted:

Only 1 (one) exposure concentration was used, 2123 mg/m^3 air [2.12 mg/l]. According to the investigators this was the maximum obtainable concentration with the equipment used. The equipment used for the generation of the "dust", the animal exposure procedure, the treatment of exhaust, the measurement of particle size distribution, the nominal concentration and gravimetric concentration calculations are described in the attached "Methods" section.

Twenty (20) animals per sex were used, the weight but not the age of the rats was provided.

The animals were exposed once for 4 hours. They were observed for mortality and signs of toxicity during exposure and at "approximately" 0.5, 1.0 and 1.1 to 3.0 hours after exposure. The animals were then observed twice at day for 14 days following exposure to the test material.

Individual body weights were taken prior to exposure and on days 3, 7 and 14 days following exposure.

All surviving animals were sacrificed at 14 days and a complete post-mortem examination was conducted on each rat

IV. Results:

No deaths were reported from the 4 hour exposure to the test compound in this study.

According to the investigators, the only compound related sign of toxicity was transient salivation observed in both males and females. The exposure technique used apparently caused ocular and nasal irritation and lacrimation. This was observed in both control and treated animals. One control rat presented with alopecia and an eye injury. Some animals showed neck edema which was apparently related to the exposure procedure.

Treated male rats gained significantly less weight than the control males. However, this cannot be considered weight loss since rats continued to gain weight over the study period and the treated male rats were heavier than control males rats at the start of the study. No effects on body weight or body weight gain were noted in the female rats (both individual animal and mean data were provided).

Post-mortem examinations conducted at 14 days showed no compound related effects. There were incidental findings of 1 control female with a white eye zone, 1 treated female with a pitted zone in the lungs, 1 treated male with dark pink lungs, another with a cortical kidney cyst, another with an enlarged kidney and another with a [illegible]

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The gravimetric mean was determined to be 2123 mg/m^3 [2.12 mg/l] (nominal concentration was 10.721 mg/m^3 [10.72 mg/l]). The mean particle size (mass median aerodynamic diameter) was found to be 4.9 um with a geometric standard deviation of 2.7 um.

Temperature range during exposure was 23.0 to 25.7°C for control and 22.5 to 24.8°C for treated animals. Relative humidity range was 15 to 34% for control and 11 to 25% for treated animals.

The LC_{50} for SENCOR® 50% Wettable Powder is greater than the gravimetric concentration of 2123 mg/m^3 [2.12 mg/l] for male and female rats.

This dose is considered the maximum obtainable concentration for the equipment used and it produces no compound related mortality.

V. Core Classification: Core-Minimum Data, although only one dose was used, it can be considered the maximum obtainable concentration.

Toxicity Category: Tox Cat III

METRIBUZIN

RIN: 3187-91

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✓ FIFRA registration data.

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I. Study Type: DNA damage/repair: Unscheduled DNA Synthesis in Primary Rat Hepatocytes (Guideline §84-2)

Study Title: Unscheduled DNA Synthesis in Rat Primary Hepatocytes
Test Article
SENCOR Technical (Metribuzin)

EPA Identification Numbers: EPA ID No. 3125-270
EPA Accession No. 262227
EPA Record No. 171481
Shaughnessy Code: 101101-4
Caswell No. 33D
Tox Branch Project No. 1775
Document No.

Sponsor: Mobay Chemical Corporation

Testing Laboratory: Microbiological Associates, Inc.
5221 River Road
Bethesda, Maryland 20816

Study Number: MA Study No. T4485.380
Mobay Toxicology Report No. 732
Mobay Ag Chem Report No. 91759

Study Date: March 26, 1986

Study Author: Rodger D. Curren, Ph.D.

Test Material: SENCOR®, Technical (also known as Metribuzin)
Lot: 77-297-50
Purity was not specified

Dosages: Metribuzin was added to test cultures at 0.007, 0.07, 0.7, 6.7, 20, 100 or 200 ug/ml in ethanol. Positive control was DMBA (from Kodak, Lot C9C) and was added at 3.0 or 10 ug/ml in DMSO. Ethanol (from Pharmco) or DMSO (from Aldrich) was added at 10 ul/ml as solvent control. WME (Williams Medium E) treated cultures served as untreated control.

Test Cultures: Rat hepatocyte cultures derived from livers of normal adult male Spraque-Dawley rats using procedure of Williams, et al. (In Vitro 13:809-817, 1977)

II. Reviewed by: Stephen C. Dapson, Ph.D. Stephen C. Dapson 1/29/87
Review Section V, Toxicology Branch/HED
Secondary Review by: Irving Mauer, Ph.D.
Mutagenicity Secondary Reviewer 1-29-87
Review Section VI, Toxicology Branch/HED
Section Head Sign Off: Quang Q. Bui, Ph.D., D.A.B.T.
Acting Section Head, R.S. V/T.B./HED Q Bui 2/3/87

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This study was designed to evaluate the ability of metribuzin to induce Unscheduled DNA Synthesis in rat primary hepatocytes as measured by autoradiographic methods.

III. Materials and Methods: A copy of the Materials and Methods section from the investigators report is appended. The following comments and highlights are noted:

Rat hepatocyte cultures were derived from livers of normal adult male Sprague-Dawley rats using the procedure of Williams, et al. (In Vitro 13:809-817, 1977). The procedure is described in attached materials and methods.

Dose levels were based on an "initial cytotoxicity test" where 10 dose levels ranging from 0.07 to 1993 ug/ml were tested in replicate cultures of HPC (rat hepatocyte cultures). For the UDS test the investigators employed 7 dose levels of Metribuzin ranging from 0.007 to 200 ug/ml (dissolved in ethanol) per plate. DMBA (dissolved in DMSO) was employed as positive control at dosages of 3 and 10 ug/ml per plate. Each test article and control dish received ^{3}H-thymidine at a final concentration of 10 uCi/ml. The investigators also used an additional 3 cultures per dose of Metribuzin as a parallel toxicity test.

The cells were treated for 18 to 20 hours (both in the initial test and the main test). Cells for the parallel toxicity test were harvested by trypsinization and analyzed for viable cell counts. Cells for the UDS assay were washed in serum free WME and fixed. These cells were further treated as decribed in the attached materials and methods for analysis of silver grains derived from exposure of the applied photographic emulsion. Slides derived from this procedure were read "blind" on an Artek Colony Counter (using random areas on each of the three coverslips per treatment, where possible). According to the investigators "The net nuclear counts were determined by counting three nucleus-sized areas adjacent to each nucleus and subtracting the average cytoplasmic count from the nuclear count".

The investigators presented the data as mean net nuclear counts with the standard deviation (s.d.). They also presented a "grand mean" and s.d. for each dose level and the percent of cells in repair (for cells with $\geq$ 5 net nuclear counts).

The criteria used by the investigators to evaluate the test results are as follows:

> "If the mean net nuclear count was increased by at least five counts over the control, the results for a particular dose level were considered significant.
>
> A test article was judged positive if it induced a dose-related response and at least one dose produced a significant increase in the average net nuclear grains when compared to that of the control.

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In the absence of the dose response, a test article which showed a significant increase in the mean net nuclear grain count in at least two successive doses was considered positive. If a test article showed a significant increase in the net nuclear grain count at one dose level without any dose response, the test article was considered to have marginal positive activity.

The test article was considered negative if no significant increase in the net nuclear grain counts at any dose level was observed."

A Quality Assurance Statement was included in this report relating to inspection dates, phases of the study inspected and report dates of the QA inspections.

IV. Results

Results of the preliminary cytotoxicity assay showed a relative toxicity (R.T.) of 100% for Metribuzin at 664 ug/ml with a R.T. of 91.1% for Metribuzin at 199 ug/ml. No toxicity was noted at doses of 6.6 ug/ml and lower (reported in attached Table 1). The investigators noted that at the 2 highest doses (664 and 1993 ug/ml), some chemical not in solution. No precipitate was noted at lower doses.

Doses chosen for the main (UDS) assay ranged from 0.007 to 200 ug/ml. The parallel cytotoxicity assay in the main study is reported in attached Table 2 (mean data were provided). A R.T. of 100% was noted at 200 ug/ml with a R.T. of 77.8% noted at 100 ug/ml of Metribuzin. No R.T. was noted at dose levels of 6.7 ug/ml and lower (the investigators determined the lower 3 doses by visual inspection). The 2 doses of DMBA of 3.0 and 10 ug/ml produced R.T.'s of 29.8% and 47.6% repectively. No toxicity was noted in the vehicle or untreated controls.

The UDS assay results are reported in attached Table 3. According to the investigators, they did not count the 0.007 ug/ml dose level since the protocol only required that 5 dose levels be counted. Based on their criteria for evaluation of test results, both doses of the positive control DMBA induced a significant increase in the average net nuclear count of silver grains. None of the doses of Metribuzin counted induced a significant increase in the mean net nuclear count of silver grains. The high dose (200 ug/ml) produced no relative survival and therefore was "too toxic to count". The percent of cells "in repair" was similar for both the solvent (ethanol and DMSO) and treated groups.

V: Conclusions

Under the conditions of this test, Metribuzin did not cause a significant increase in the Unscheduled DNA Synthesis (UDS) as measured in this study, whereas the positive control did increase UDS.

Core Classification: Acceptable

METRIBUZIN

RIN: 3187-91

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005709

I. Study Type: Gene Mutation in Mammalian Cells in vitro:
CHO/HGPRT Assay
(Guideline §84-2)

Study Title: CHO/HGPRT Mutation Assay in the Presence and Absence of Exogenous Metabolic Activation
Test Article
SENCOR Technical (Metribuzin)

EPA Identification Numbers: EPA ID No. 3125-305
EPA Accession No. 262227
EPA Record No. 171482
Shaughnessy Code: 101101-4
Caswell No. 33D
Tox Branch Project No. 1776
Document No.

Sponsor: Mobay Chemical Corporation

Testing Laboratory: Microbiological Associates, Inc.
5221 River Road
Bethesda, Maryland 20816

Study Number: MA Study No. T4485.332
Mobay Toxicology Report No. 733
Mobay Ag Chem Report No. 91760

Study Date: March 26, 1986

Study Author: Li Lillian Yang, Ph.D.

Test Material: SENCOR®, Technical (also known as Metribuzin)
Lot: 77-297-50
Purity = 92.6%

Dosages: Metribuzin was added to cells at levels of 1000, 900, 800, 700 or 600 ug/ml in the non-activated study and at levels of 200, 175, 150, 100 or 50 ug/kg in the presence of a S-9 activation system.
Ethyl methanesulfonate (EMS, Aldrich, lot 0422 BM) was used as positive control in the non-activated study at a concentration of 0.2 ul/ml.
Benzo(a)pyrene (BaP, Sigma, lot 13F-9006) was used as positive control in the activated study at a concentration of 4 ug/ml.
Solvent control was acetone (Fisher, lot 851079) at the "same concentration as test article groups".

Test Cultures: CHO-K_1-BH_4 cells (Dr. Abraham Hsie, Oak Ridge National Laboratories, Oak Ridge, TN).

Metabolic Activator: S-9, 9000 x g supernatant of an Arochlor-1254 induced Fischer 344 rat liver homogenate.

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II Reviewed by: Stephen C. Dapson, Ph.D. Stephen C. Dapson 1/29/87
Review Section V, Toxicology Branch/HED
Secondary Review by: Irving Mauer, Ph.D. 1/29/87
Mutagenicity Secondary Reviewer
Review Section VI, Toxicology Branch/HED
Section Head Sign Off: Quang Q. Bui, Ph.D., D.A.B.T.
Acting Section Head, R.S. V/T.B./HED 2/3/87

This study was designed to assess the mutagenic potential of Metribuzin based on its ability to induce forward mutation at the HGPRT locus of CHO cells.

III. Materials and Methods: A copy of the Material and Methods section from the investigators report is appended. The following comments and highlights are noted:

The S-9 metabolic activation system was prepared from livers of adult male Fischer rats weighing 200-250 gm who received a single IP injection of Arochlor-1254 at a dosage of 500 mg/kg body weight. The procedure is described on attached materials and methods. According to the investigators "Each bulk preparation of S-9 is assayed for its ability to metabolize 2-aminoanthracene and 7,12 dimethyl-benz(a)anthracene to forms mutagenic to Salmonella typhimurium TA100".

The investigators performed a range finding study based on colony forming efficiency. They exposed CHO cells to either solvent alone (control) or to 9 concentrations of Metribuzin ranging from 0.1 ug/ml to 1000 ug/ml for 5 hours 37±1°C in the presence and absence of metabolic activation.

The mutation assay used published methodologies of Mechanoff, R., O'Neill, J.P., and Hsie, A.W. (Quantitative analysis of cytotoxicity and mutagencity of benzo(a)pyrene in mammalian cells (CHO/HGPRT), Chem. Biol. Interactions 34:1-10, 1981) and O'Neill, J.P., Brimer, P.A., Machanoff, R., Hirsch, G.P., and Hsie, A.W. (A quantitative assay of mutation induction at the hypoxanthine-guanine phosphoribosyl transferase locus in Chinese hamster ovary cells (CHO/HGPRT system): Development and definition of the system, Mutation Research 45:91-101, 1977).

Exponentially growing CHO-K_1-BH_4 cells were plated in specially prepared Ham's F-12 medium (see attached materials and methods). The time the cells were first exposed to Metribuzin was designated as day 0. The plated cells were exposed in duplicate to 4 concentrations of Metribuzin for 5 hours at 37±1°C. This was done for both the metabolically activated and non-activated study. After treatment "the cells were subcultured to assess cytotoxicity and to initiate the phenotypic expression period".

For cytotoxicity evaluation, the replicates from the primary mutation study for each treatment group were pooled and subcultured in triplicate, and after a further 7 to 10 day incubation the cells were then fixed, stained and counted.

For mutant phenotype expression, the replicates from the primary mutation study for each treatment group were pooled and subcultured in duplicate and further subcultured at 2-3 day intervals for the 7-9 day expression period. The cells were then selected for the TG-resistant phenotype by pooling and replating (in quintuplicated) from each treatment group. Also, for cloning efficiency determinations, cells were plated in triplicate. After a 7-10 day incubation period, the colonies were fixed, stained and counted for cloning efficiency and mutant selection.

The cytotoxicity was expressed relative to solvent control, as the relative cloning efficiency.

The mutation frequency (MF) was calculated by dividing the total number of mutant colonies counted by the number of plates selected, corrected for the cloning efficency of the cells. This cloning efficiency is determined prior to mutant selection. The MF is expressed as TG-resistant mutants per 10^6 number of clonable cells. If no mutant colonies are observed, mutation frequencies will be expressed as less than the frequency obtained with one mutant colony. If doses give $\leq$ 10% relative survival they were not considered as valid data points for mutation frequencies.

The investigators feel that calculation of mutagenic response based only on a "fold increase" in mutation frequency is not a reliable measure for some loci with very low spontaneous mutation frequencies. They decided that "For assays characterized by a wide degree of variation in the frequency of spontaneous mutants found in the negative or solvent controls, a confidence interval can be calculated by the application of a one-sided Student's t test ($p \leq .05$) from the historic background mutation frequency" (Gupta, R.S. and Sing, B., 1982, Mutagenic reposes of five independent genetic loci in CHO cells to a variety of mutagens: development and characteristics of a mutagen screening system based on selection for multiple drug resistant markers, Mutation Research 94:449-466). They decided that the mutagenic response after treatment will only be considered significant if the treatment mutation frequency is increased above the negative controls by at least 8.7 mutants/10^6 clonable cells and at least twice that of the solvent and untreated controls.

According to the investigators, "The assay will be considered positive in the event a dose-dependent increase in mutation frequency is observed with one or more of the five concentrations tested inducing a mutation frequency which is at least twice that of the solvent control, and also is increased above that of the solvent control and the untreated control by at least 8.7 mutants/10^6 clonable cells. The study will be considered suspect if there is no dose response but one or more dose induce a mutation frequency which is considered significant. The assay will be considered negative if none of the doses tested induce a mutation frequency which is considered significant."

For a valid test the investigators stated that: "The cloning efficiency of the solvent and untreated controls must be no less that 50%. The spontaneous mutatic frequency in the solvent and untreated controls must fall within the range of 0-20 mutants per 10^6 clonable cells. And further: "The positive control must induce a mutation frequency at least three times that of the solvent control."

A Quality Assurance Statement was included in this report relating to inspection dates, phases of the study inspected and report dates of the QA inspections.

IV: Results

The range finding study conducted to determine doses to be used in the CHO/HGPRT assay determined the cloning efficency after exposure to either the solvent alone or to one of nine concentrations of Metribuzin ranging from 0.1 ug/ml to 1000 ug/ml both in the presence and absence of S-9 activation. The results are reported in attached Table 1. It was noted that Metribuzin without metabolic activation produced relative cloning efficiency comparable to solvent control at all dose levels tested. Metribuzin in the presence of S-9 activation produced relative cloning efficiency of 96, 100, 95, 91, 82, 76, 42, 0 and 1 % for the 0.1, 0.3, 1, 3, 10, 30, 100, 300 and 1000 ug/ml respectively. The dose levels chosen for the primary study were 600, 700, 800, 900 and 1000 ug/ml witnout metabolic activation and 50, 100, 150, 175 and 200 ug/ml in the presence of S-9 activation.

The cytotoxicity results from the 5 hour treatment of CHO cells (in the presence and absence of S-9 activation) in primary study are reported in Table 2. For the non-activated study the doses of 600, 700, 800, 900 and 1000 ug/ml of Metribuzin produced relative cloning efficency of 118, 80, 68, 55 and 61% respectively. For the metabolically activated study the doses of 50, 100, 150, 175 and 200 ug/ml produced relative cloning efficiencies of 123, 63, 40, 0.4 and <0.4% respectively. The positive control produced relative cloning efficiencies of 66 and 34 for the nonactivated and metabolically activated studies respectively. Similar effects on survival were seen in the concurrent cytotoxicity studies with the mutation assay (reported in attached Tables 3 and 4).

The CHO/HGPRT mutation assay in the absence of metabolic activation is reported in Table 3. The solvent control produced 8 thioguanine-resistant mutant colonies which calculates to a mutation frequency of 10.13 mutants per 10^6 clonable cells. The doses of Metribuzin of 600, 700, 800, 900 and 1000 ug/ml showed a total number of mutant colonies of 3, 4, 7, 0 and 1 (giving mutation frequencies of 4.55, 4.55, 9.21, <1.28 and 1.30 mutants per 10^6 clonable cells). Therefore, no significant effect was noted. The positive control showed a total of 171 mutants with a mutation frequency of 259.09 mutants per 10^6 clonable cells.

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The CHO/HGPRT mutation assay in the presence of S-9 activation is reported in Table 4. The solvent control produced 4 mutant colonies which gives a mutation frequency of 4.60 mutants per 10^6 clonable cells. The high dose (200 ug/ml) was too toxic to carry out "mutant expression and mutant selection". The doses of Metribuzin of 50, 100, 150 and 175 ug/ml showed a total number of thioguanine-resistant mutant colonies of 9, 1, 6 and 4 (giving mutation frequencies of 9.28, 1.09, 6.32, and 4.82 mutants per 10^6 clonable cells). None of the dosage levels tested showed levels significantly above that of the solvent control. The positive control showed a total of 343 mutants with a mutation frequency of 581.36 mutants per 10^6 clonable cells.

V: Conclusions

Under conditions of this study, Metribuzin is negative in the CHO/HGPRT mutation assay.

Core Classification: Acceptable.

METRIBUZIN

RIN: 3187-91

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