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TXR-4622

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

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OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Guthion: Miscellaneous Toxicity Data
Accession Numbers 255242 and 255245 CASWELL #374

FROM: George W. Robinson, D.V.M. George W. Robinson 8/16/85
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Thirty individual toxicity studies were submitted in a brochure entitled "Guthion Human Safety Data, October 1984," Accession No. 255245. Copies of several of the same studies were submitted in a brochure entitled "Human Safety of Guthion, Supplement No. 2, September 25, 1978, to brochure entitled: Guthion Toxicology, Dated December 13, 1968," Accession No. 255242.

Toxicological reviews of the newly submitted toxicity data follow.

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1. The Acute Oral Toxicity of Guthion® Technical, Benzazimide and Methyl Benzazimide to Rats, by C.R. Crawford and R. H. Anderson, Chemagro Division of Baychem Corporation, July 23, 1974; Accession Number 255245.

Test Material: Guthion Technical, 99.0% ai

Test Animals: Adult Sprague-Dawley male and female rats weighing 245 to 305 g and 190 to 236 g, respectively; 4 rats/sex were assigned to each of 4 dose levels.

Administration:

Guthion Technical was dissolved in dimethyl sulfoxide (DMSO) to obtain dosage volumes equivalent to 0.1 percent body weight. Animals were fasted 24 hours prior to initiation of the study. Four males and 4 females received single oral doses of Guthion at each of 4 dose levels: 2, 4, 8, and 16 mg/kg bwt. Rats were observed for 14 days.

Results:

Any and all mortality in rats occurred between 12 and 80 minutes posttreatment.

Conclusion:

Oral LD_{50} values for Guthion dissolved in DMSO were calculated to be as follows:

Males	5.6 mg/kg bwt (95% CL = 3.5 to 9.2)
Females	6.4 mg/kg bwt (95% CL = 3.1 to 12.9)

Classification: Core-Minimum Data; Category I.

2. The Acute Oral Toxicity of GUTHION®, Benzazimide and Methyl Benzazimide to Fasted and Nonfasted Rats Using CMC as the Excipient, by D.W. Lamb and R.H. Anderson, Chemagro Division of Baychem Corporation, September 4, 1974; Accession Number 255245.

Test Material: Guthion Technical - 99.0% ai

Test Animals: Adult Sprague-Dawley male and female rats weighing 223 to 355 g and 188 to 260 g, respectively; 4 fasted and nonfasted rats/sex assigned to each of 4 dose levels.

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Administration:

Guthion was suspended in a 2 percent aqueous solution of carboxymethylcellulose (CMC) to obtain a dosage volume equivalent to 0.1 percent body weight. Four fasted and 4 nonfasted males received single oral doses of Guthion at each of 4 dose levels: 8, 16, 32, and 64 mg/kg bwt. Fasted and nonfasted females received Guthion similarly at dose levels of 4, 8, 16, and 32 mg/kg bwt.

Results:

The symptoms of rats dosed with Guthion were typical of cholinesterase inhibition. Any and all mortality in rats occurred within 1 day of dosing.

Conclusion:

Oral LD_{50} values for Guthion suspended in a 2 percent aqueous solution of CMC were calculated to be as follows:

Nonfasted male rats	19 mg/kg bwt
Fasted male rats	19 mg/kg bwt
Nonfasted female rats	10 mg/kg bwt
Fasted female rats	16 mg/kg bwt

Classification: Core-Minimum Data; Category I.

3. R 1582 (Gusathion M Active Ingredient) - Acute Toxicity Studies, by F. Mihail, Bayer AG Institut fur Toxikologie, Report No. 7618, June 15, 1978; Accession Number 255245.

Test Material: R 1582 (Gusathion M active ingredient), technically pure grade (91.6%)

Test Animals:

a) Male and female Wistar albino rats weighing 170 to 210 g.

b) Male Beagle dogs weighing 12.7 to 16.2 kg.

3.1 Acute Oral Toxicity of R 1582 to Rats

Administration:

Fifteen male rats (fasted for 16 hours) received single doses of R 1582 emulsified in distilled water and Cremophor EL by gavage in a volume of 1.0 ml/100 g bwt at each of the following dose levels: 1.0, 2.5, 3.5, 4.0, 5.0, 6.0, 7.5, and 10 mg/kg bwt. Fifteen female rats were dosed similarly at dose levels of 1.0, 2.5, 3.5, 5.0, 5.5, 6.0, and 7.5 mg/kg bwt. All rats were observed for 14 days.

Results:

Symptoms typical of erratic cholinesterase activity, e.g., salivation, dyspnea, depression, muscle twitching, and clonic cramps began within 5 to 20 minutes after dosing and persisted for a maximum of 24 hours. Any and all deaths occurred with 1 hour posttreatment.

Conclusion: Oral LD_{50} for R 1582 emulsified in distilled water and Cremophor EL are as follows:

Male rats	4.6 mg/kg bwt
Female rats	4.4 mg/kg bwt

Classification: Core-Minimum Data; Catagory I.

3.2 Acute Oral Toxicity of R 1582 to Male Dogs

Administration:

R 1582 emulsified in distilled water and Cremophor EL was administered by gavage in a volume of 2 ml/kg bwt to one male dog each at dose levels of 1.0, 2.5, and 5.0 mg/kg bwt; 2 male dogs received 10.0 mg/kg bwt of R 1582 similarly.

Results:

One dog at the high dose level vomited the R 1582 about 2 hours posttreatment. No deaths occurred at any dose level; there were no overt symptoms.

Conclusion:

LD_{50} was > 10 mg/kg (no mortality at highest dose).

Classification: Core-Supplementary Data (too few dogs per dose, and dose levels too low).

3.3 Acute Dermal Toxicity of R 1582 to Rats

Administration:

Hair was clipped from the dorsal surfaces of male and female rats on the day before treatment. Five rats/sex were assigned to each of 2 dose groups which received R 1582 at 100 and 500 mg/kg bwt. Four additional groups of 10 rats/sex received R 1582 at dose levels of 1000, 1500, 2500, and 5000 mg/kg bwt. A 25 percent emulsion of R 1582 in water and Cremophor EL was applied to the intact dorsal skin of rats at 100 mg/kg bwt. A paste of R 1582 using 10 to 20 drops of Cremophor EL was applied to the intact dorsal skin at dose

levels of 500 mg/kg and above. Treated areas were wrapped with adhesive plaster sleeves. After a contact time of 24 hours the adhesive plaster sleeves were removed and R 1582 was washed from the skin with soap and water. All surviving rats were observed for 14 days posttreatment.

Results:

Symptoms observed were salivation, dyspnea, and clonic cramps with onset 1 day posttreatment and persisting for 2 to 11 days. Dose-related deaths occurred in male and female rats at dose levels of 1000 mg/kg bwt and above between 2 and 10 days posttreatment.

Conclusion:

LD_{50} (14 days) was determined to be 2500 to 5000 mg/kg bwt in male and female rats.

Classification: Core-Minimum Data; Category III.

4. R 1582 (Azinphos-Methyl, the Active Ingredient of GUTHION®) Study of the Acute Oral and Dermal Toxicity to Rats by K.G. Heimann and D. Lorke, Bayer AG Institut fur Toxikologie, Mobay AgChem No. 82383, June 30, 1982, Accession Number 255245.

Test Material: R 1582 (azinphos-methyl, the active ingredient of Guthion), Batch No. 79-R-225-42, Purity 88.9 percent.

Test Animals: Male and female Wistar albino rats of the WISP strain (SPF-CPB) housed under standardized conditions with feed and water ad libitum.

4.1 Acute Oral Toxicity, Fasted and Fed Male Rats

Administration:

R 1582 emulsified in Cremophor EL/distilled water (5 drops/10 ml) was administered by gavage in a volume of 1 ml/100 g bwt. Fasted rats were dosed as follows: 20 males received a single dose of 5.0 mg/kg bwt; 3 groups of 10 males each received single doses of 6.3, 6.7, and 8.0 mg/kg bwt, respectively. Four groups of 10 fed male rats each received single doses of 10.0, 12.5, 16.0, and 20.0 mg/kg bwt. Rats were observed for 14 days.

Results:

Symptoms which occurred as early as 10 minutes in the lethal range and up to 8 days in survivors included: dyspnea,

lethargy, piloerection, salivation, spastic staggering gait, sternal recumbency, and tremors. Dose-related deaths occurred in all dose groups within 30 minutes in fasted males and within 1 hour in fed male rats.

Conclusion:

LD_{50} (14 days) were calculated to be as follows:

6.7 mg/kg bwt in fasted male rats, and
12.8 mg/kg bwt in fed male rats.

Classification: Core-Minimum Data; Catagory I

4.2 Acute Dermal Toxicity, Rats

Administration:

The skin of the backs of male and female rats was clipped free of hair on the day before treatment. R 1582 was emulsified in Cremophor EL/distilled water (5 drops/10 ml) and applied to the intact dorsal skin at doses ranging from 60 to 400 mg/kg bwt as follows:

Dose mg/kg	Number of rats per group	
	Males	Females
63	-	5
100	5	5
160	10	10
200	10	-
250	5	5
315	10	-
400	10	-

Treated skin areas were covered with bandages wrapped around the trunk. After a contact time of 24 hours, bandages were removed and R 1582 was washed from the skin with soap and water. Rats were observed for 14 days posttreatment.

Results:

Dose-related deaths occurred at doses of 100 mg/kg bwt and above in females and 200 mg/kg bwt and above in males between 1 and 7 days posttreatment.

Conclusion:

LD_{50} (14 days) values were calculated to be 200 to 250 and 155 mg/kg bwt in males and females, respectively.

Classification: Core-Minimum Data; Category I.

5. R 1582 (Azinphos-Methyl, the Active Ingredient of Guthion®) Study of the Irritant Effect on the Skin and Mucous Membranes (Eye), by J. Thyssen and D. Lorke, Bayer AG Institut fur Toxikologie, Mobay AgChem No. 87434, October 19, 1981; Accession Number 255245.

Test Material: R 1582 (azinphos-methyl, the ai of Guthion), Batch No. 230105019, purity 92.4 percent.

Test Animals: Male and female New Zealand white rabbits weighing 3 to 4 kg.

5.1 Test on the Rabbit Skin

Administration:

"The experiments were conducted in accordance with the recommended guidelines of the U.S. Department of Agriculture, Federal Register, 38 (187):27019 (1973). The duration of exposure was 24 hours."

Results:

No irritant effect on the skin of rabbits was recorded for R 1582.

Conclusion:

Toxicological evaluation of the irritant effect of R 1582 on the skin of rabbits could not be made due to the absence of descriptive experimental methods from the study report.

Classification: Invalid.

5.2 Test on the Rabbit Eye

Administration:

"The experiments were conducted in accordance with the recommended guidelines of the U.S. Department of Health, Education and Welfare, Federal Register, 37 (83):8535 (1972)."

Results:

No irritant effect of the mucous membranes of the eyes of rabbits was recorded for R 1582 after short-term (5 minutes) and long-term (24 hours) exposures.

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Conclusion:

Toxicological evaluation of the irritant effect of R 1582 on the mucous membranes of the eyes of rabbits could not be made due to the absence of descriptive experimental methods from the study report.

Classification: Core-Invalid.

6. Anticholinesterase Toxicity Studies with Guthion, Phosdrin, Disyston, and Trithion in Human Subjects, by J.A. Rider, J.I. Swader and E.J. Puletti, Federation Meeting, Atlantic City, NJ, April 10, 1972, Mobay AgChem No. 29672; Accession Number 255245.

Conclusion:

There were no data presented from which an adequate toxicological evaluation could be made.

Classification: Invalid.

7. The Effect of Daily Oral Administration of GUTHION® to Cattle at Doses of 5 and 15 ppm for 30 Days, by C.R. Crawford and R.H. Anderson, Chemagro Division of Baychem Corporation Research and Development, Report No. 35408, January 8, 1973; Accession Number 255245.

Test Material: Guthion (54.1% Wettable Powder, Batch No. 2090269).

Test Animals: Holstein bull calves each weighing approximately 124 kg.

Administration:

Each of 3 groups of 3 calves received the equivalency of 0, 5, and 15 ppm ai in the diet once daily for 30 days by oral administration in gelatin capsules. The control group (0 ppm) received capsules containing 50% Wettable Powder consisting of inert ingredients only. Blood samples were collected 1 week pretreatment and weekly thereafter. Daily feed consumption per group and weekly individual body weights were recorded. Whole blood cholinesterase activity was determined on all frozen blood samples at the end of the 30-day study.

Results:

No significant effect on cholinesterase activity, feed consumption, and growth rates were observed in male calves fed Guthion for 30 days at 5 and 15 ppm.

Conclusion:

An adequate toxicological evaluation of this study could not be made for the following reasons:

1. The actual dosage of the active ingredient of Guthion is uncertain. The report states that:

> "It is assumed that the cattle will consume 3% of their body weight in feed. At this rate 1 ppm Guthion in the diet is equivalent to 0.030 mg/kg/day. At this equivalency 5 and 15 ppm are equal to 0.150 and 0.450 mg Guthion/kg/day, respectively. All doses are expressed in terms of active ingredient."

No mention was made as to the actual quantity of Guthion dispensed in the capsules. Why the dosages were calculated in ppm consumed in the assumed daily diet rather than mg/kg bwt is unknown.

2. Daily pen (group) feed consumption could not be related directly to individual body weights of the calves.

Classification: Core-Supplementary Data.

8. E 1582 R (Azinphos-Methyl, the Active Ingredient of Guthion) Study of the Toxicity of Its Pyrolytic Products, by J. Thyssen, Bayer AG Institute fur Toxikologie, Bayer Report No. 8914, January 30, 1980; Accession Number 255245.

Conclusion:

This was a translation of "Summary" only. No data were presented from which a toxicological evaluation could be made. Also, this type of study would not have satisfied a toxicity data requirement.

Classification: Invalid.

9. R 1582 (Azinphos-Methyl, the Active Ingredient of Guthion) Study of the Toxicity to Rats Particularly in Regard to Cholinesterase Activity, by R. Eiben, W. Schmidt and E. Loeser, Bayer AG Institut fur Toxicologie, Bayer Report No. 11813, May 18, 1983; Accession Number 255245.

Conclusion:

This was a translation of "Summary" only of a 28-day feeding study as a range-finding test for a 2-year study. No data were presented from which a toxicological evaluation could be made.

Classification: Invalid.

10. The Acute Oral Toxicity of Guthion® 2S in Rats, by D.L. Nelson, Chemagro Agricultural Division, Mobay Chemical Corporation, Report No. 66514, August 15, 1978; Accession Number 255245.

Test Material: Guthion 2S, Batch No. 7030094, Formula No. 011013 containing 2 lb (22%) active ingredient per gallon. Dilutions were made in Carbowax 500 (polyethylene glycol).

Test Animals: Male and female albino Sprague-Dawley strain rats (Holtzman Co., Madison, WI) weighing 282 to 450 g and 168 to 248 g, respectively. Rats were housed 5/cage under standard controlled laboratory conditions with feed and water ad libitum except during the 18-hour fasting period just prior to dosing.

Administration:

Dosage groups of 10 rats were dosed orally with a volume equivalent to 0.5 percent of their body weight at the following levels:

Males	15, 22, 32, 47, and 69 mg/kg bwt
Females	10, 15, 22, 32, 47, and 69 mg/kg bwt

All rats were observed daily for 14 days. Individual body weights were recorded on days 0, 7, and 14. All surviving rats were sacrificed on day 14.

Results:

Signs of toxicity generally began in 1/2 hour and lasted 4 to 24 hours and included: salivation, lacrimation, hypoactivity, tremors, convulsions, and diarrhea. Deaths occurred in treated rats in 1/2 hour to 4 hours. Necropsies of rats that died and were sacrificed revealed no gross lesions which were compound-related.

Conclusion:

LD_{50} values and 95 percent confidence limits for Guthion 2S in rats were calculated to be as follows:

Males	37 (30 to 47) mg/kg bwt
Females	21 (16 to 26) mg/kg bwt

Classification: Core-Minimum Data; Catagory I.

11. Acute Oral Toxicity of Guthion 2L to Rats, by D.L. Nelson, Mobay Chemical Corporation, Corporate Toxicology Department, Stanley Research Center, Report No. 68028, July 18, 1979; Accession Number 255245.

Test Material: Guthion 2L; Batch No. 9030068, Formula No. 011016 containing 2 lb (22%) active ingredient per gal. Dilutions were made in Carbowax 400 (polyethylene glycol).

Test Animals: Male and female Spraque-Dawley derived rats (Sasco, Inc., Omaha Nebraska) weighing 274 to 400 and 179 to 235 g, respectively. Rats were housed 5/cage under standard controlled laboratory conditions with feed and water ad libitum except during the 19-hour fasting period just prior to dosing.

Administration:

Dosage groups of 10 rats/sex were dosed orally with a volume equivalent to 0.5 percent of their body weight at levels of 22, 32, 47, 69, 102, and 220 mg/kg bwt. All rats were observed over a 14-day period for mortality and signs of toxicity. Individual body weights were recorded on days 0, 7, and 14. Necropsies were performed on all animals that died and on all surviving rats on day 14.

Results:

Signs of toxicity generally began in 1/2 hour and lasted 4 to 24 hours and included: salivation, lacrimation, tremors and convulsions. Deaths occurred in treated rats in 1/2 to 48 hours. Necropsies revealed no compound related gross lesions.

Conclusion:

LD_{50} values and 9.5 percent confidence limits for Guthion 2L in rats were calculated to be as follows:

Males	75 (53 to 106) mg/kg bwt
Females	55 (44 to 70) mg/kg bwt

Classification: Core-Minimum Data; Catalog II.

12. Acute Oral Toxicity of Guthion™ 50% Wettable Powder, by D.L. Nelson, Mobay Chemical Corporation, Corporate Toxicology Department, Stanley Research Center, Report No. 68029, July 16, 1979; Accession Number 255245.

Test Material: Guthion, Batch No. 9143520, Formula No. 011011, 50% ai Wettable Powder. Dilutions were made in distilled water.

Test Animals: Male and Female Sprague-Dawley derived rats (Sasco, Inc., Omaha, NE) weighing 284 to 382

and 204 to 260 g, respectively. Rats were housed 5/cage under standard controlled laboratory conditions with feed and water ad libitum except during the 18- to 20-hour fasting period just prior to dosing.

Administration:

Dosage groups of 10 rats/sex were dosed orally with a volume equivalent to 0.5 percent of their body weight at levels of 8, 16, 32, 64, and 128 mg/kg bwt. All rats were observed over a 14-day period for mortality and signs of toxicity. Individual body weights were recorded on days 0, 7, and 14. Necropsies were performed on all rats that died and on all surviving rats on day 14.

Results:

Signs of toxicity generally began in 1/2 hour and lasted 4 to 48 hours and included: salivation, lacrimation, tremors and convulsions. Deaths occurred in rats in 1 to 48 hours. Necropsies revealed no compound-related gross lesions.

Conclusion:

LD_{50} values and 95 percent confidence limits for Guthion 50% Wettable Powder were calculated to be as follows:

Males	48 (38 to 61) mg/kg bwt
Females	34 (26 to 45) mg/kg bwt

Classification: Core-Minimum Data; Catagory I.

13. Acute Dermal Toxicity of Guthion® 2S Emulsifiable to Rabbits, by D.L. Nelson, Mobay Chemical Corporation, Chemagro Agricultur Division, June 30, 1978, Report No. 66250; Accession Number 255245.

Test Material: Guthion 2S Emulsifiable, Batch No. 7030094, Formula No. 011013, containing 2 lbs (22%) active ingredient per gal.

Test Animals: Male and female New Zealand white rabbits weighing 2.04 to 2.79 kg and 2.09 to 2.88 kg, respectively. Rabbits were housed singly in cages under standard controlled laboratory conditions with feed and water ad libitum.

Administration:

The skin of the backs of male and female rabbits was clipped free of hair on the day before treatment. Intact

skin was then abraded with a bristle brush. Guthion 2S was applied to abraded skin of four rabbits/sex at the following dose levels:

Males	200, 400, 800, and 1600 mg/kg bwt
Females	200, 400, 520, 676, 879, and 800 mg/kg bwt

Treated sites were covered with plastic and taped securely. Rabbits remained under restraint for 24 hours after which wrappings were removed and Guthion was wiped from test sites with a soft dry cloth. Rabbits were observed daily for mortality and signs of toxicity for 14 days. Individual body weights were recorded on days 0, 7, and 14. Necropsies were performed on all rabbits that died and on all surviving rabbits sacrificed on day 14. Mortality was analyzed according to the method of Carrol S. Weil, Biometrics 8(3), 9/52.

Results:

Signs of toxicity occurred within 1 to 24 hours and included: salivation, diarrhea, decreased activity, exophthalmos, lethargy, ataxia, tremors and convulsions. Deaths occurred from 18 hours to 6 days post-treatment. Necropsies revealed no compound related gross lesions.

Conclusion:

LD_{50} values and 95 percent confidence limits for Guthion 2S Emulsifiable on abraded skin were calculated to be as follows:

Males	504 (310 to 820) mg/kg bwt
Females	568 (472 to 682) mg/kg bwt

Classification: Core-Minimum Data.

Tox. Category: II

14. The Acute Inhalation Toxicity of Guthion 2S to Rats, by D.L. Nelson, Mobay Chemical Corporation, Chemagro Agricultural Division, Report No. 66157, May 26, 1978; Accession No. 255245.

Test Material: Guthion 2S. Batch No. 7030094, Formula No. 011013.

Test Animals: Male and female Sprague-Dawley strain rats from Holtzman Co., Madison, WI. Rats were housed 5/cage/sex in a standard controlled laboratory environment with feed and water ad libitum.

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Administration:

Ten rats/sex/dose group were exposed to undiluted Guthion 2S in a dynamic flow inhalation chamber (Kimmerle and Eben, Arch. Toxikol. 30:115, 1973) at 856, 1146, and 1471 µg of formulation per liter of air for 60 minutes. A group of 10 male rats was also exposed to 2343 µg/liter of air. After 15 and 45 minutes of exposure, one 4-liter sample of air was collected from the chamber and analyzed for actual concentration. The chamber was sampled after 30 minutes of exposure for determination of droplet size in the aerosols. All rats were observed for mortality and signs of toxicity over a 14-day period. Necropsies were performed on all rats that died and on all surviving rats sacrificed after 14 days posttreatment.

Results:

Signs of toxicity occurred at all dose levels from 1/2 hour exposure to 5 days posttreatment and included: hypoactivity, salivation, lacrimation, diarrhea, exophthalmos, muscle fasciculations, piloerection, tremors, and convulsions. Most deaths occurred while the animals were in the chamber; a few others occurred within 1 day postexposure. Necropsies revealed no Guthion-related gross lesions.

Conclusion:

LC_{50} values and 95 percent confidence limits for 60 minutes inhalation exposure of rats to aerosols of Guthion were calculated to be as follows:

Females: 1080 (659 to 1771) µg/liter
Males: 1365 (941 to 1979) µg/liter

Classification: Core-Minimum Data.

Tox. Category: II

15. The Eye and Dermal Irritancy of Guthion 2S to Rabbits, by D.L. Nelson, Mobay Chemical Corporation, Chemagro Agricultural Division, Report No. 66124, May 24, 1978; Accession No. 255245.

Test Material: Guthion 2S, emulsifiable, containing 2 lbs (22%) ai/gal; Batch No. 7030094, Formula No. 011013.

Test Animals: New Zealand white rabbits (Small Stock, Inc., Pea Ridge, AR). Rabbits were caged individually under standard controlled laboratory conditions with feed and water ad libitum.

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a. Eye Irritation

Administration:

The eyes of nine rabbits were examined with fluorescein under ultraviolet light for evidence of current defects or irritation prior to treatment. Left eyes were then treated with 0.1 ml of undiluted Guthion; right eyes served as controls. Treated eyes of 3 rabbits were washed with 200 ml of lukewarm water 45 seconds posttreatment. Eyes were examined on days 1, 2, 3, 4, and 7 and later if eye lesions persisted.

Results:

Reactions of the eye to Guthion 2S were scored according to J.H. Draize. Primary irritation score (mean) at 72 hours was 5.33/110. Slight redness, chemosis, and discharge persisted in 2/3 rabbits with washed eyes and in 2/6 rabbits with nonwashed eyes on day 7 posttreatment.

Conclusion:

Undiluted Guthion 2S produced mild irritation to the conjunctivae of rabbits at 72 hours post-treatment. However, slight irritation persisted in the treated eyes of 4/9 rabbits on day 7.

Mear PIS = 5.33/110 @ 72 nours

Classification: Core-Minimum Data.

Tox. Category: II

b. Dermal Irritation

Administration:

Hair was closely clipped from the backs and sides of six rabbits. Two test sites were chosen and delineated for use. One test site was abraded with a 19 gauge needle. One-half (0.5) ml of undiluted Guthion 2S was applied to each of the two test sites under a 1-inch^2 gauze pad on each rabbit. A plastic sheet was wrapped around the trunk of each rabbit which was restrained with a plastic collar. Collars, wrappings, and gauze were removed 24 hours later and Guthion was washed from test sites with acetone. Skin of tests were evaluated, and again at 72 hours, by the method of Draize.

Results:

Well-defined erythema was present on both test sites in all rabbits at 24 hours. Slight erythema persisted at 72 hours on both test sights in 4/6 rabbits. Edema was absent.

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Conclusion:

Guthion 2S is a mild irritant to the skin of rabbits. Primary Irritation Index = 1.33/8.0.

Classification: Core-Minimum Data.

Tox. Category: IV

16. Eye and Dermal Irritancy of Guthion® 50% Wettable Powder, by E.J. Hixon, Mobay Chemical Corporation, Corporate Toxicology Department, Stanley Research Center, Report No. 68336, November 15, 1979; Accession No. 255245.

Test Material: Guthion 50% Wettable Powder, Batch No. 9143520, Formula No. 011011.

Test Animals: New Zealand white rabbits from Small Stock, Inc., Pea Ridge, AR were caged individually with feed and water ad libitum under standard controlled laboratory conditions.

a. Eye Irritation

Administration:

The eyes of nine rabbits were examined with fluorescein under ultraviolet light for preexisting defect or irritation. One hundred mg of test material was placed in the left eye of all rabbits. After 45 seconds, the treated eyes of 3 rabbits were washed with 200 ml of lukewarm water. Treated eyes were examined on days 1, 2, 3, 4, and 7 posttreatment.

Results:

Slight to moderate erythema occurred in all treated eyes by 24 hours with one exception; there was no adverse reaction in the washed eye of 1 rabbit. Chemosis was present in the unwashed eyes of 3/6 rabbits. Discharge appeared in 1/3 washed and 5/6 unwashed eyes. Corneal opacity and iritis was present in the unwashed treated eye of 1/6 rabbits but was clear by day 4.

Conclusion:

Guthion 50% Wettable Powder is a moderate irritant to the eyes of rabbits.

Classification: Core-Minimum Data.

Tox. Category: III

b. Dermal Irritation

Administration:

Hair was closely clipped from the backs and sides of six rabbits. Four test sites were chosen and delineated for use. Two test sites were abraded with a 19 gauge needle. Plastic collars were placed on the rabbits. One-half (0.5) gram of test material moistened with physiological saline was applied to each of four test sites under a 1-inch^2 gauze pad. A plastic sheet was wrapped around the trunk of each rabbit. Collars, wrappings, and gauze were removed 24 hours later and Guthion was wiped from test sites with a damp cloth. Test sites were evaluated 24 and 72 hours posttreatment according to J.H. Draize.

Results:

Slight erythema and slight edema was present at 24 hours at test sites on both intact and abraded skin in 5/6 rabbits. No evidence of skin irritation was present at 72 hours post-treatment.

Conclusion:

Guthion 50% Wettable Powder was a very mild skin irritant in rabbits.

Primary irritation index = 0.71/8.0.

Classification: Core-Minimum.

Tox. Category: IV

17. Subchronic Inhalation Toxicity of Azinphos-Methyl to Rats, by G. Kimmerle, Institut fur Toxikologie der Bayer AG, Arch. Toxicol. 35:83-89 (1976); Accession No. 255245.

Test Material: Azinphos-methyl (Gusathion, Guthion, DBD, Bayer 17147) technical grade, a brown waxy solid, was diluted in a 1:1 mixture of ethanol and polyethylene glycol 400.

Test Animals: Male and female SPF Wistar Rats (150-170g and 130-150g resp) from F. Winkelmann, Versuchstierzucht GmbH und Ko. Rats were caged individually under standard controlled laboratory conditions with feed and water ad libitum.

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Administration:

Four groups of 10 rats/sex were exposed in dynamic inhalation chambers to mean aerosol concentrations of azinphos-methyl in the air of 0, 0.195, 1.24 and 4.72 mg/m^3, respectively. Controls inhaled only the solvent mixture. Rats were exposed to the above for 6 hours 5 days a week over a 12-week period.

Rats were observed daily and weighed weekly. Plasma and erythrocyte cholinesterase activity was determined after 2, 4, 6, 8, 10, and 12 weeks. Hematological and clinical laboratory determinations (GOT, GTP, AP, urea, creatinine, bilirubin) were performed after 12 weeks inhalation, just prior to sacrifice. Gross necropsies were performed on all rats. The weights of thyroid, thymus, heart, lungs, liver, spleen, kidneys, adrenals, and gonads were recorded. These same organs were examined histologically. Aerosol droplet size was ascertained using a cascade impactor; 97 percent of the droplets had a diameter of 1 $\pm$ 0.5 μ.

Results:

Male and female rats tolerated inhalation exposure to azinphos-methyl at concentrations up to 4.72 mg/m^3 with no significant changes in appearance and behavior. However, a significantly lower mean body weight gain was detected only in male rats in the high dose group. Plasma and erythrocyte cholinesterase activity was inhibited 30 to 40 percent at 4.72 mg/m^3 but unaffected at lower concentrations. Cholinesterase activity of the brain was not affected at any level.

There were no significant alterations in hematological values, serum enzyme activities (GOT, GPT, AP), levels of creatinine, bilirubin, and urea or in the composition of urine. No gross or histopathological lesions could be related to inhalation exposure to azinphos-methyl.

Conclusion:

Azinphos-methyl at 4.72 mg/m^3 5 days/week for 12 weeks caused significant inhibition of cholinesterase activity in plasma and erythrocytes.

NOEL (ChE) = 1.24 mg/m^3 (both sexes)
NOEL = (Other than ChE) 1.24 mg/m^3 (males - lower bwt gain)

Classification: Core-Minimum Data.

18. R 1582 Studies for Embryotoxic and Teratogenic Effects On Rabbits Following Oral Administration, by L. Machemer, Bayer AG Institut fur Toxikologie, Report No. 5455, June 3, 1975; Accession No. 255245.

Test Material: R 1582, the active ingredient of Gusathion insecticide/acaricide, 92.4 percent.

Test Animals: Himalayan rabbits, sexually mature males and females weighing 2 to 2.5 kg supplied by Dr. Karl Thomae GmbH, Biberach. Rabbits were caged singly under standard controlled laboratory conditions with feed and water ad libitum.

Procedure:

Each doe was mated with one buck until two copulations were observed; this day was considered gestation day 0. Eleven to 12 fertilized does per group received daily doses of R 1582 from gestation day 6 through 18 by gavage at the following levels: 0, 0.3, 1, and 3 mg/kg bwt. R 1582 was diluted in a 0.5 percent aqueous Cremophor emulsion and administered at a constant volume of 5 ml/kg to each dose group. Control does received the same volume of the Cremophor emulsion only.

All pregnant does underwent Caesarean section on gestation day 29. All fetuses were sexed and thoroughly examined for external malformations and alterations. Average fetal weight per litter and litter weights were determined and stunted fetuses weighing less than 25 g were counted. Autopsies were performed on all fetuses and the abdominal and thoracic organs were examined for visceral anomalies. The head of each fetus was removed, fixed and prepared for sectioning. All fetuses were eviscerated, the bodies were cleared with dilute KOH, stained with Alizarin Red S, and examined for skeletal anomalies. The numbers of fetuses examined in control, low, mid and high dose groups were 79, 64, 66 and 77, respectively.

Results:

All does survived the 29-day gestation period with no adverse effects on physical appearance and general behavior. There were no significant differences between treated and control groups mean body weight gains, fertilization or pregnancy rates, mean numbers of implantations, mean numbers of live fetuses, mean fetal and placental weights, fetal sex ratios, and resorption rates. There were no visceral or skeletal anomalies which could be ascribable to treatment with R 1582.

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Conclusion:

Oral administration of R 1582 to pregnant rabbits of up to and including 3 mg/kg bwt/day from gestation day 6 through 18 produced no maternal toxicity, embryotoxicity, fetotoxicity or teratogenicity.

NOEL = 3 mg/kg bwt/day for maternal toxicity, embryotoxicity, fetotoxicity, and teratogenicity

Classification: Core-Supplementary Data.

Note: The highest dose level should induce some overt maternal toxicity such as slight weight loss, but not more than 10 percent maternal deaths. Also, feed consumption should be monitored and recorded.

19. R 1582 Dominant Lethal Study on Male Mouse To Test For Mutagenic Effects, by B. Herbold, Bayer AG Institut fur Toxikologie, Report No. 8425, June 7, 1979; Accession No. 255245.

Test Material: R 1582, Batch 230705148/201-300, 92.3 percent, the insecticidal active ingredient of Gusathion M, common name: azinphos-methyl.

Test Animals: Mice, NMRI strain, 8 to 12 weeks old, supplied by S. Ivanovas GmbH, Kisslegg/Allgau. Males weighed 31 to 43 g; females weighed 28 to 33 g. Animals were housed under standard controlled laboratory conditions with feed and water ad libitum. There were 50 males each in test and control groups; test and control groups contained 598 females each.

Procedure:

The test dose was chosen on the basis of a range-finding study with an acute oral NOEL of 2.5 mg/kg bwt. Males in the single test group received a single oral dose of 4 mg/kg bwt of test material in 0.5 percent Cremophor EL emulsion in a volume of 10 ml/kg bwt. Control group males received an equivalent volume of the vehicle only. Each dosed male was immediately caged with one untreated virgin female which commenced a series of 12 uninterrupted 4-day mating periods lasting a total of 48 days. At the end of each 4-day mating period, the mated female was removed and caged singly, and replaced by another untreated virgin female. Midway through a mating period was considered the time of conception. Uterine contents of each female were examined 14 days later and total implants, live implants, dead implants, and corpora lutea were counted and recorded. These parameters were analyzed by a 2-factor analysis of variance and the Kolmogorov-Smirnov (non-parametric) test.

Results:

Male mice treated with a single oral dose of 4 mg/kg bwt R 1582 exhibited no signs of toxicity and did not differ from controls in behavior, physical appearance, survival and fertility.

Preimplantation and postimplantation losses were similar in females bred to R 1582 dosed males and vehicle control males. No significant differences between test groups were detected by statistical analysis of parameters tested (corpora lutea, total implants, viable implants, dead implants).

Conclusion:

No mutagenic effect was detected from a single oral dose of R 1582 (azinphos-methyl) at 4 mg/kg bwt in the dominant lethal test conducted on male mice.

Classification: Unacceptable: 1) only 1 dose level; 2) no clinically adverse effects; 3) no evidence of absorption and transport to germ cells; 4) no positive control.

20. Micronucleus Test on Mouse to Evaluate R 1582 for Potential Mutagenic Effects, by B. Herbold, Bayer AG Institut fur Toxikologie, Report No. 8521, July, 19, 1979; Accession No. 255245.

Test Material: R 1582, 230705148/201-300, the insecticidal active ingredient of Gusathion M; common name: azinphos-methyl; purity of 92.3 percent

Positive Control: Treniman®, a known mutagen and a cytostatic drug.

Test Animals: Male and female mice, NMRI strain, 8 to 12 weeks old, supplied by S. Ivanovas GmbH, Kisslegg. Mice weighed 22 to 32 and were housed under standard controlled laboratory conditions with feed and water ad libitum.

Procedure:

Mice were randomly assigned to each of 4 groups of 10 (5/sex). Each of two groups received R 1582 at 2.5 and 5.0 mg/kg bwt, respectively, in a 0.5 percent Cremophor EL emulsion orally by stomach tube. A vehicle negative control group received only the emulsion orally. The positive control group received 0.125 mg/kg bwt Trenimon by intraperitoneal injection. All mice received a second identical

dose of the appropriate substance 24 hours after the initial. Six hours after the second dose, the mice were sacrificed and femoral bone marrow smears were prepared and processed for evaluation.

Microscopic evaluation of 1,000 polychromatic erythrocytes per mouse was performed to determine the incidence of cells with micronuclei. The number of normochromatic erythrocytes per 1,000 polychromatic erythrocytes were counted to determine the ratio of polychromatic erythrocytes to normochromatic erythrocytes.

The criterion for mutagenic effect in the micronucleus test, a somatic mutagenicity test system in vivo, is an increase in the incidence of micronucleated polychromatic erythrocytes (due to chromosome breaks or disturbances of the mitotic spindle apparatus).

The Wilcoxon ranking test was used for statistical analysis of results.

Results

There were no significant differences between R 1582-treated groups at doses up to and including 2 x 5 mg/kg bwt orally and the vehicle negative control group for the following parameters: the incidence of micronucleated polychromatic erythrocytes, the number of micronucleated normochromatic erythrocytes, and the ratio of polychromatic erythrocytes to normochromatic erythrocytes. The incidence of micronucleated polychromatic erythrocytes in the positive control (Trenimon-treated) mice was significantly higher than that in the vehicle negative control group. Also, there was a significant reduction in the ratio of polychromatic erythrocytes to normochromatic erythrocytes in the positive control group compared to the vehicle negative control group; this is an indication of depression of erythropoiesis.

Conclusion:

R 1582 (azinphos-methyl), at dose levels up to and including 2 x 5 mg/kg bwt orally was not mutagenic in the micronucleus test as conducted in mice which responded with a positive reaction to the known mutagen, Trenimon.

Classification: UNACCEPTABLE: 1) insufficient dosage; 2) no clinical effects at HDT; 3) no evidence material was absorbed in sufficient amounts to transport to target tissue.

21. Salmonella/Microsome Test for Determination of Point Mutations, by B. Herbold, Bayer AG Institut fur Toxikologie, Report No. 7965, December 4, 1978; Accession No. 255245.

Test Material: R 1582, Batch 230705148/201-300, purity 92.3 percent; active ingredient of the insecticide Guthion; common name: azinphos-methyl.

Positive Controls: Endoxan (Asta), Batch 7320; its active ingredient cyclophosphamide is a known promutagen.

Trypaflavin (Roth), Batch 0282995; the active ingredient of Panflavin and Rivanol is a frameshift promutagen.

Solvents:

Dimethylsulfoxide (DMSO) for R 1582 and trypaflavin

Demineralized water for Endoxan

Bacterial Tester Strains:

Auxotrophic histidine-dependent mutants of Salmonella typhimurium LT2: strains TA 98, TA 100, TA 1535, and TA 1527.

S-9 Mix:

The S-9 fraction (stored at -80 °C) was the 9,000 x g supernatant from homogenized livers of adult male Sprague-Dawley rats which received a single intraperitoneal injection of Aroclor 1254 at a dose of 500 mg/kg bwt 5 days before sacrifice. The S-9 mix was freshly prepared by slowly thawing aliquots of the S-9 fraction and adding the appropriate co-factors.

Procedure:

Frozen bacterial cultures of each strain were thawed, suspended in nutrient broth and incubated at 37 °C for 24 hours. Four agar plates were prepared for each dose of each substance tested for each bacterial tester strain to determine mutant counts. R 1582 was tested at doses of 0 (negative control), 4, 20, 100, 500, and 2500 µg/plate. The negative control received only the solvent, DMSO. Endoxan, positive control, was tested at a dose of 725 µg/plate on strains TA 100 and TA 1535 only. The other positive control, trypaflavin, was tested at 250 µg/plate on strains TA 98 and TA 1537 only. All groups were tested with S-9 mix. The highest dose of each test substance was also tested without S-9 mix. The plates were incubated for 48 hours at 37 °C prior to making total bacterial and mutant counts.

Criterion:

The rate of reverse mutation to prototrophy is determined in treated and control groups. A dose-related increase in the number of mutations to a level double that of the negative control, obtained with at least one bacterial tester strain, is regarded as a positive result.

Results:

R 1582 produced no bacteriotoxic effect at doses up to and including 2500 ug/plate. Total bacterial counts were not significantly different between R 1582-treated and negative control groups. No dose-related increase in the number of mutations was detected in any of the four bacterial tester strains.

Mutation counts in the positive controls (Endoxan and trypaflavin) were 4 to 36 times that of the vehicle negative controls in the respective bacterial tester strains with S-9 mix. Also, trypaflavin was bacteriotoxic to strain TA 1537 (bacterial growth inhibition). In plates without S-9 mix, mutation counts were not significantly different in positive and negative controls.

Conclusion:

R 1582, at levels up to and including 2500 ug/plate, did not produce any bacteriotoxic or mutagenic effects in the four bacterial tester strains used in the Salmonella/microsome test. The significant mutagenic effects produced by the positive control substances attest to the great sensitivity of the system and the activity of the S-9 mix in this test.

Classification: UNACCEPTABLE as comprehensive assay for bacterial reverse mutation:

(1) Test substances was not tested up to cytotoxic concentrations or levels of insolubility (at least to the limit dose = 5000 ug/plate).

(2) inadequate controls.

22. Mutagenicity Evaluation of R 1582 (Azinphos-methyl) in the Reverse Mutation Induction Assay with Saccharomyces Cerevisiae Strains S138 and S211a, by A.J.W. Hoorn, Litton Bionetics, the Netherlands, Genetics Assay No. E-9108, June 1983; Accession Number 255245.

Test Material: R 1582, Batch No. 230205060, purity 91.1 percent.

Positive Control Material:

a. Quinacrine Mustard, 10 ug/ml in dimethylsulfoxide (DMSO), in the nonactivation assay with yeast strain S138.

b. Ethylmethanesulfonate 1 percent in the nonactivation assay with yeast strain S211a.

c. Sterigmatocystin, 5 ug/ml in DMSO for activation assays with both strains.

Negative Solvent Control:

Dimethylsulfoxide (DMSO) at 100 ul per 3 ml

S-9 Metabolic Activation System:

The commercially purchased S-9 fraction was a 9000 x g supernatant from the homogenate of the liver of adult male Sprague-Dawley rats previously treated with Aroclor 1254. The S-9 mix was prepared by adding the appropriate co-factors to the S-9 fraction.

Yeast Tester Strains:

Two methionine auxotrophs of Saccharomyces cerivisiae - strain S138, a frameshift mutant, and strain S211a, a base-pair substitution mutant.

Procedure:

The reverse mutation induction assay was conducted on R 1582 at 6 doses ranging from 33.3 to 10,000 ug/ml. Doses used in this assay were chosen on the basis of results of a preliminary toxicity study conducted on the test material at 14 doses ranging from 1.22 to 10,000 ug/ml with strain S211a. R 1582 was not toxic to strain S211a at any of the doses tested.

The following is a verbatim quote from the protocol from pages 9 and 11 of the study report:

> "Stocks of the yeast strains S138 and S211a are maintained as isolates at 4 °C on plates containing yeast complete media. Working stock suspensions of the strain are obtained from the late log phase cultures grown at 30 °C in yeast extract peptone. The selective media consisted of yeast minimal medium. Survival was determined using yeast complete medium. The overlay agar consisted of 0.6% purified agar with 0.1M NaCl and supplemented with 10 mg/l of methionine . . . ."

"(1) Nonactivation Assay

To a sterile vial, the following were added:

(a) 2 ml of yeast cells (grown overnight) at 1 - 2x10^8 cells/ml.
(b) 0.90 - 0.97 ml of phosphate buffer (pH 7.4)
(c) 0.03 - 0.10 ml of a solution of the test compound to give the appropriate dose.

The above mixture was incubated for 3 hours at 30 °C in a rotary shaker. After incubation, the suspension was used to assay for revertants and cell survival as follows:

Aliquots of the suspension were placed in 2 ml molten overlay (at 45 °C) supplemented with methionine (at 10 mg/l) and poured on methionine deficient media.

Aliquots of an appropriate dilution of the suspension were placed in 2 ml of overlay and poured onto yeast complete plates.

The plates were incubated for approximately 3 days for population counts and 5 to 7 days for revertant counts and then counted and recorded.

(2) Activation Assay

The activation assay was run concurrently with the nonactivation assay. The only difference was the addition of 0.90 to 0.97 ml of the S-9 mix to the vials in place of the phosphate buffer which was added to the nonactivation assays. All other details were similar to the procedure for nonactivation assays."

Negative solvent controls were employed with and without metabolic activation for both tester strains. Positive control compounds known to selectively revert each strain to methionine prototrophy were also assayed concurrently with the test material.

Results:

The results of the reverse mutation induction assay were summarized in tabular form. There were no raw data and no explanation or demonstration of the derivation of numerical values as presented. However, based on the data at hand, it appears that test results were as follows:

R 1582 (azinphos-methyl), at 6 threefold serial concentrations from 33.3 to 10,000 µg/ml in

cultures of S. cerivisiae strains S138 and S21la, did not produce a significant increase in the number of methionine revertants at any dose level. The frequency of revertants were similar in cultures treated with R 1582 and the solvent alone (negative control) both with and without metabolic activation.

The positive controls (quinacrine mustard for S138 and ethylmethanesulfonate for S21la) produced significant increases in the frequency of methionine revertants in the nonactivation assays. Sterigmatocystin, the positive control for both strains in the assays with metabolic activation, did not produce any significant increases in revertant counts.

Conclusion:

The results as presented in this report are inconclusive for the following reasons:

1. There is no indication of how the dose levels (concentrations of test material) were determined.

2. Admitted deviations from the protocol regarding aliquots and dilution of cell suspensions could not be interpreted.

3. Derivation of numerical values as presented in tabular form is incomprehensible and no raw data given, or how values were determined.

4. No mention is made of the number of replicate plates per dose level or of the reproducibility in one or more additional tests.

5. The highest dose (10,000 ug/ml) of R 1582 was lower than a toxic concentration which was predetermined in a preliminary toxicity test. This may well account for the lack of increases in revertant counts over the dose range used in this test.

6. The positive control, sterigmatocystin @ 5 ug/ml, for both strains of S. cerivisiae produced no mutagenic effects under existing test conditions with S-9 metabolic activation. Therefore, the activation assay test system appeared to be nonfunctional. In table 3 there is a notation which states "There is no known positive control compound that works consistently in the activation assay."

Classification: Unacceptable.

23. Evaluation of R 1582 C.N. Azinphos-Methyl in the Primary Rat Hepatocyte Unscheduled DNA Synthesis Assay, by B. C. Myhr, Litton Bionetics, Inc., Maryland, Project No. 20991, November 1983; Accession No. 255245.

Test Material: R 1582 (Batch No. 230 205 060, Purity 91.1%) C.N. Azinphos-methyl.

Positive Control Material: 2-acetyl aminofluorene @ 0.05 ug/ml

Negative Solvent Control: 1 percent dimethylsulfoxide (DMSO).

Media: Williams' Medium E (WME) with 5 percent fetal bovine serum for establishment of primary hepatocyte cultures and WME with 1 percent fetal bovine serum for the unscheduled DNA synthesis (UDS) assay.

Experimental Design:

Serial dilutions of R 1582 were made in DMSO to prepare stock solutions ranging from 100,500 to 2.51 ug/ml. Appropriate stock solutions were further diluted 1:100 in WME containing 1 percent fetal bovine serum to obtain test concentrations of 100.5, 50.3, 25.1, 10.1, 5.0, 2.5, 1.0, 0.5, and 0.25 ug/ml.

Fresh hepatocytes were obtained from an adult male Fischer 344 rat by perfusion of the liver in situ with a collagenase solution. Monolayer cell cultures were established in 35 mm culture dishes containing plastic coverslips and 3 ml of WME with 5 percent fetal bovine serum. Cell cultures were incubated at 37 °C for an attachment period of 1.5 hours. The UDS assay was initiated approximately 2.5 hours later by replacing the media with the appropriate test concentrations of R 1582 containing 1 uCi/ml ^{3}H-thymidine. Each test concentration and negative and positive controls in WME were deposited onto five cell cultures. After an 18-hour treatment period, 2 of the 5 cell cultures were washed twice with WME and used for viability counts and evaluation of toxicity; the remaining 3 cell cultures were washed with WME containing 1 mM thymidine and further processed for UDS evaluation.

Viable cell counts of treated cultures were determined by trypan blue exclusion technique; cell survival was estimated relative to the negative solvent control.

Coverslips with labeled cells were fixed, dried, mounted on glass slides (cell side up), dipped in Kodak NTB2 emulsion and dried. The slides were then stored in light-proof boxes containing a dessicant for 7 to 10 days, developed in D19,

fixed, and stained with Williams' modified hematoxylin and eosin technique. Nuclear grain counts were determined from the microscopic field as displayed on the video screen of an automatic counter. The mean number of grains in three nuclear-sized cytoplasmic areas adjacent to the nucleus subtracted from the nuclear grain count yielded the net nuclear grain count.

Results:

The 100.5 ug/ml dose was lethal leaving 8 dose levels ranging from 50.3 ug/ml to 0.25 ug/ml for UDS evaluation. The 50.3 ug/ml dose was toxic (cell survival was 21.5%). Survival was 59.4 percent at 25.1 ug/ml but similar to that of the negative solvent control at 10 ug/ml and lower doses. The positive control, 0.05 ug/ml of 2-AAF was nontoxic.

At 50.3 ug/ml nuclear grain counts were made for 75 cells from one culture; insufficient viable cells were present in the 2 other cultures. At least 150 cell nuclei were scored at all lower doses.

Nuclear labeling in R 1582-treated cell cultures was essentially the same as that recorded for the negative solvent control cultures. A dose-related response was not observed. Considerable nuclear labeling occurred in positive control cultures which received 0.05 ug/ml of 2-AAF, a nontoxic dose. The primary rat hepatocytes were, therefore, responsive to the detection of UDS in this assay system.

Conclusion:

R 1582 (azinphos-methyl), at 8 concentrations ranging from a cytotoxic level 50.3 to 0.25 ug/ml, did not produce any significant degree of nuclear labeling which was indicative of UDS in primary rat hepatocytes. Nuclear labeling in R 1582-treated cultures was similar to that observed in negative solvent control cultures. 2-Acetyl aminofluorene, the positive control, at 0.05 ug/ml did induce UDS in primary rat hepatocytes, an indication that this assay system was adequate.

Classification: Acceptable.

24. R 1582 C.N. Azinphos-Methyl Pol Test on E. coli to Evaluate for Potential DNA Damage, by B. Herbold, Bayer AG Institute of Toxicology, Report No. 12478, February 22, 1984; Accession No. 255245.

Test Material: R1582, Batch 230 205 060, 91.1 percent ai, common name: azinphos-methyl.

Negative Control: Chloramphenicol (Boehringer, Mannheim), Batch 2123391080.

Positive Control: Methylmethanesulfonate (Merck, Darmstadt), Batch 1171879.

Toxicological evaluation of the Pol Test on E. coli is not possible due to the paucity of information provided in the report as specified below:

1. There is inadequate description of the E. coli tester strains and no supportable scientific justification for their use in mutagenicity testing.

2. A clear, brief, complete description of preparation of the S-9 fraction and S-9 mix was not provided.

3. There are serious deficiencies in the description of methods (experimental design or procedure) used in both the conduct of the study and presentation and evaluation of results, including the following:

   a. dose selection

   b. dosing procedure

   c. lack of raw data

   d. analysis of data

   e. criteria for acceptance of assay

   f. assay evaluation criteria

Classification: Unacceptable.