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

007153

APR 28 1989

MEMORANDUM

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

SUBJECT: Methyl Parathion: Absorption, Distribution, Excretion and Metabolism in Rats.

TO: Dennis Edwards PM-12
Registration Division (H7505C)

FROM: K. Clark Swentzel K. Clark Swentzel 4/25/89
Acting Section Head
Toxicology Branch II (HFAS)
HED (H7509C)

THRU: Marcia van Gemert, Ph.D. Marcia van Gemert 4/27/89
Acting Branch Chief
Toxicology Branch II (HFAS)
HED (H7509C)

EPA ID No.: 4787-4
MRID Acc. No.: 410014-07
Project No.: 9-0951
Caswell No.: 372
Registrant: A/S Cheminova

Requested Action:

Review subject study.

Conclusion:

Methyl parathion (U-^{14}C-phenyl-labeled) was administered orally to male and female rats in order to follow the absorption, distribution, excretion and metabolism of the radio-labeled molecule after single oral administration of the test material at 2 dose levels and after repeated oral (14x) pre-treatment using the non-radioactive test material followed by single oral administration of the ^{14}C-labeled material.

The data showed that a large proportion of the administered radioactivity was absorbed from the gastro-intestinal tract and excreted in the urine. Within 8 hours, 61.8 to 94.0% of the radioactivity was excreted in the urine of male and female rats. After 48 hours the corresponding values were 75.7 to 99.2% and, at this interval, 3.2 to 9.3 of the administered radioactivity was excreted in feces. Only negligible amounts of administered radioactivity was found in organs, tissues, blood (<0.1 - 1.0%) and expired air (<0.01%). Total average recoveries for both sexes ranged from 95.6 to 104.2%.

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Repeated oral (14x) administration of non-labeled methyl parathion had no apparent effect on the rate of absorption and excretion of the ^{14}C-labeled material or on the levels of residual radioactivity in organs/tissues and blood.

Similar metabolic patterns were found in urine and feces regardless of the dose of test material administered.

Seven metabolites were detected in urine, 5 of which were characterized. The characterized metabolites represented up to 94.7% of the radioactivity recovered in urine. The two major urinary metabolites were the sulphate conjugate of para-nitrophenol (60.6 to 79.3% of urinary radioactivity) and the glucuronide conjugate of para-nitrophenol (up to 15% of urinary radioactivity). Three minor metabolites detected were para-nitrophenol, P-O-desmethyl-paraoxon-methyl and P-O-desmethyl-parathion-methyl; small amounts of the latter 2 metabolites were also found in feces. Although only 2 of the 6 metabolites detected in fecal extracts were characterized, the total radioactivity recovered in feces was quite low (1.0 to 4.2% of administered dose). The parent compound was not detected in either urine or feces.

Core classification: minimum

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Reviewed by: K. Clark Swentzel K. Clark Swentzel 4/25/89
Section II, Toxicology Branch II (H7509C)
Secondary Reviewer: Marcia van Gemert, Ph.D. M. van Gemert 4/27/89
Acting Branch Chief, Toxicology Branch II (H7509C)

DATA EVALUALTION REPORT

STUDY TYPE: Metabolism in Rats TOX CHEM NO.: 372

MRID NO.: 410014-07

TEST MATERIAL: Unlabeled and ^{14}C labeled(phenyl)--- 0,0-dimethyl-0-4-nitrophenyl-phosphorothioate

SYNONYMS: Methyl-parathion

STUDY NUMBER: 090876

SPONSOR: Bayer AG and A/S Cheminova

TESTING FACILITY: R C C Umweltchemie AG
CH-4452 Itingen/Switzerland

TITLE OF REPORT: ^{14}C-Parathion-Methyl: Absorption, Distribution, Excretion and Metabolism after Single and Repeated Oral Administration to Rats

AUTHOR(S): A. Van Dijk

REPORT ISSUED: September 6, 1988

CONCLUSIONS:

Methyl parathion (U-^{14}C-phenyl-labeled) was administered orally to male and female rats in order to follow the absorption, distribution, excretion and metabolism of the radio-labeled molecule after single oral administration of the test material at 2 dose levels and after repeated oral (14x) pre-treatment using the non-radioactive test material followed by single oral administration of the ^{14}C-labeled material.

The data showed that a large proportion of the administered radioactivity was absorbed from the gastro-intestinal tract and excreted in the urine. Within 8 hours, 61.8 to 94.0% of the radioactivity was excreted in the urine of male and female rats. After 48 hours the corresponding values were 75.7 to 99.2% and, at this interval, 3.2 to 9.3 of the administered radioactivity was excreted in feces. Only negligible amounts of administered radioactivity was found in organs, tissues, blood (<0.1 - 1.0%) and expired air (<0.01%). Total average recoveries for both sexes ranged from 95.6 to 104.2%.

Repeated oral (14x) administration of non-labeled methyl parathion had no apparent effect on the rate of absorption and excretion of the ^{14}C-labeled material or on the levels of residual radioactivity in organs/tissues and blood.

Similar metabolic patterns were found in urine and feces regardless of the dose of test material administered.

Seven metabolites were detected in urine, 5 of which were characterized. The characterized metabolites represented up to 94.7% of the radioactivity recovered in urine. The two major urinary metabolites were the sulphate conjugate of para-nitrophenol (60.6 to 79.3% of urinary radioactivity) and the glucuronide conjugate of para-nitrophenol (up to 15% of urinary radioactivity). Three minor metabolites detected were para-nitrophenol, P-O-desmethyl-paraoxon-methyl and P-O-desmethyl-parathion-methyl; small amounts of the latter 2 metabolites were also found in feces. Although only 2 of the 6 metabolites detected in fecal extracts were characterized, the total radioactivity recovered in feces was quite low (1.0 to 4.2% of administered dose). The parent compound was not detected in either urine or feces.

Core classification: minimum

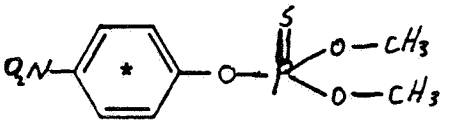
Quality Assurance: a signed and dated statement was included

Test material:

Unlabeled: Batch no.: APF 24108550; Purity: 98.1%; Aggregation state: white solid; Stability in water: at least 2 hrs; Storage conditions: +4° C, in the dark

Labeled: Code: KML 270; Specific radioactivity: 57.6 mCi/g (2.13 MBq/mg); Radiochemical purity: 99.1%; Stability in vehicle (corn oil): stability was demonstrated by TLC-analyses of radioacitivity

Structural formula/position of label (*):



Dosing solutions:

Unlabeled: The material was initially dissolved in toluene and subsequently prepared for oral administration in corn oil (procedures are described in detail on Appended page 1).

Labeled: This material was also initially dissolved in toluene and prepared in corn oil for oral administration at 2 dosage levels (0.5 and 2.5 mg/kg). A final specific radioactivity of 12.1 mCi/g was obtained for the high dose solution (procedures are described in detail on Appended page 1).

Test animals:

Male and female Wistar rats, KFM-WIST outbred, SPF-quality; age not given; body weights were approximately 180 - 220 g; individually identified by ear tags; 7-day acclimation period (including 2-3 days in metabolism cages)

Husbandry:

Housing: Groups of 2-3 rats were in Makrolon cages with standard soft wood bedding during acclimatization. Other caging accomodations varied with the treatment regimen.

Diet: Pelleted 343-Kliba rat maintenance diet (18 g/rat/day)

Water: Tap water provided ad libitum

Health: The status of treated animals was checked at daily intervals.

Methods:

Pre-test:

Three rats/sex were administered unlabeled test compound in corn oil by oral intubation at the high dose (2.5 mg/kg) selected for the metabolism study. This test was performed to assure the investigator that this dosage level would not induce mortalities.

Results:

No mortalities occured during the 48-hr observation period. It was indicated that the males (number not given) "showed some sedative and spasmodic signs during the first 4 hours after administration."

Metabolism Study:

The stated objectives of this study were to follow the absorption, distribution, excretion and metabolism of the ^{14}C-labeled molecule after single oral administation of the test article at 2 dose levels and after repeated oral (14x) pre-treatment using the non-radioactive test article followed by a single dosage of the radioactively labeled compound to male and female rats. Since the test compound has low solubility in water (55 mg/l at 20°C) and high toxicity, it was not administered intravenously.

The levels of radioactivity appearing in expired air (high-dose males only), urine, feces, blood and organs/tissues were followed in separate experiments as indicated below. The investigator characterized 6/7 radioactive fractions detected in urine and 2/6 fractions found in feces.

The methods used in the separate experiments in this study results are included below.

Balance study in males receiving the high-dose level:

Five male rats were administered single oral doses (2.5 mg/kg) of ^{14}C-labeled test material and placed in glass metabolism cages which were ventilated with about 600 ml air/minute. The outcoming air was passed through a trapping system containing a mixture of ethanolamine/2-methoxy-ethanol followed by a dry-ice/ethanol cooled trap containing 2-methoxy-ethanol. The solution of the CO_2 trapping system was changed 8, 24 and 48 hours after administration.

Urine was collected into dry-ice/ethanol cooled tubes at 4, 8, 24, 32 and 48 hrs after adminsitration. The readioactivity was directly determined by liquid scintillation counting of subsamples. Thereafter, the remaining urine was stored at -20°C until further analysis.

Feces was collected at room temperature at the same time intervals noted for urine, homogenized wet after addition of water and the radioactivity was then determined by combustion of subsamples. Samples collected from 8-24 hrs were pooled.

After 48 hrs, the anesthetized animals were killed by exsanguination. All but 0.5 - 1.0 was centrifuged and the plasma was decanted. The following organs were removed: heart, lung, liver, stomach, spleen, intestinal tract (with contents), kidney, gonads (ovary/uterus or testicle), muscle (femur), bones, brain, skin (back region), fat and residual carcass (including skin). The radioactivity in organs/tissues, blood and plasma was determined after digestion of subsamples with tissue solubilizer; the remaining samples were stored at -20°C until further analysis. The digestive tract and the carcass were homogenized and subsamples were combusted to determine radioactivity; bones were directly combusted.

The cages were washed with water and acetone and radioactivity was determined by combustion.

Balance study in males receiving the low dose (0.5 mg/kg) and females receiving the high and low doses:

Five males and five females were each administered 0.5 mg/kg and five females were each given 2.5 mg/kg ^{14}C-labeled test material as single doses via oral intubation. The experimental period (48 hrs) as well as the sampling and analytical procedures were described for the previous experiment, however, expired air was not analyzed in this experiment. Feces samples collected between 8-24 hrs for males, 8-32 hrs for low dose females and 4-24 hrs for high dose females, were pooled.

Excretion study after repeated (14x) oral administration of unlabeled test material followed by a single oral dose of ^{14}C-labeled compound:

Five males and five females received 14 daily doses (0.5 mg/kg) of unlabeled methyl parathion followed by one dose (0.5 mg/kg) of ^{14}C-labeled compound; all administrations were by oral intubation. The post treatment interval (48 hrs) as well as the sampling and analytical procedures were the same as those used in the previous experiment. Feces samples collected between 4-24 hrs were pooled for both males and females.

Sample preparation and characterizeation of radioactivity:

The metabolic pattern (number and quantity) was investigated in urine and feces. Isolated compounds were compared with reference compounds by means of high performance liquid chromatography (HPLC) and thin-layer chromatography (TLC). In addition, selected metabolites were mixed with glucuronidase and arylsulphatase and the resulting radioactive moieties were compared with reference compounds by chromatographic methods.

Urine:

The radioactivity in urine was determined directly by liquid scintillation counting. Based on the radioactivity found, urine aliquots (20% by volume) were pooled from samples collected between 0-24 hrs in each experiment.

The metabolite pattern was established by one-dimensional TLC with ethyl acetate/n-propanol/water (75/45/15) as the solvent system (SS 2). In addition, two-dimensional TLC (solvent systems: 2 and trichloromethane/methanol/water[SS 6: 60/40/4]) was used to determine if metabolites previously isolated by one-

TLC could be further separated into additional fractions. Additionally, an aliquot (9.0 ml) of the urine pool from high dose males was lyophilized and dissolved in 3.0 ml methanol (recovery = 94.5%). This solution was used for TLC with SS 2. After development, the radioactive zones were scraped off, homogenized and put into a glass column; the radioactivity was eluted with 30 ml acetone/water (8+2, v/v). The investigator indicated that more than 93% of the radioactivity was recovered by this method. Following elution, separate fractions were concentrated at 50°C by rotary vacuum evaporation. The partially solid material was dissolved in approximately 1.0 ml of a mixture of acetone, methanol and a small amount of water (respective proportions not given); the stated recovery was 92-100%.

Evidently, urine metabolites isolated in the other experiments were characterized by means of co-chromatography with reference compounds and by the addition of glucuronidase and sulphatase.

Feces:

Feces were homogenized wet after addition of water and the radioactivity was determined by combustion of subsamples. Aliquots of feces (50% of the weight of each sample) were pooled as previously indicated.

Aliquots of feces were extracted 4 times with acetone/water (8+2, v/v) and 2 times with methanol/water (8+2, v/v). The remaining feces were air-dried, homogenized and the non-extractable radioactivity was determined by combustion of subsamples.

For characterization, aliquots of extractables were subjected to ultrafiltration (recovery = 75-93%) and the ultrafiltrate was concentrated at 50°C by means of rotary evaporation. Residual radioactivity was dissolved in methanol/water and the solution was used for one dimensional TLC and if possible, isolated compounds were compared with reference compounds.

Analytical methods:

The methods used in this study for quantitation and characterization of various radioactive fractions are described in detail on Appended pages 2 - 9.

Results:

Balance data and excretion patterns:

Single oral administration (Appended pages 10 - 13):

The major proportion of administered radioactivity was excreted via the urine in both males and females at both dose levels: 99.2 and 92.8% in high and low dose males, respectively; corresponding values in females were 75.7 and 79.9%. Excretion in urine was also rapid: 61.8% (high dose females) to 94.0% (high dose males) of administered radioactivity was excreted in 8 hrs following administration.

The average excretion of administered radioactivity in feces ranged from 3.2% (low dose females) to 9.3% (high dose females).

Group values for total excreted radioactivity (urine, feces and cage wash) ranged from 95.2% (low dose females) to 103.9% (high dose males).

Trace amounts of radioactivity were detected in the intestinal tract (up to 0.3% in high dose females), in the residual carcass (up to 0.3% in all groups), in organs/tissues (up to 0.1% in low dose males) and in blood (up to 0.1% in high dose females). Less than 0.01% of administered radioactivity was found in expired air (measured only in high dose males).

Total recovery of administered radioactivity (group means) ranged from 95.6% (low dose females) to 104.2% (high dose males).

Repeated oral administration (Appended pages 14 and 15):

As in animals administered single doses of methyl parathion, the major route of excretion in animals receiving multiple doses was via the kidneys; 92.9 and 91.8% of administered radioactivity was excreted in the urine of males and females, respectively. Also, the rate of urinary excretion was comparable to that observed in rats receiving single doses.

Excretion of radioactivity in feces was 5.3 and 3.4% of the administered dose in males and females, respectively.

Total excreted radioactivity (urine, feces and cage wash) averaged 99.6 and 99.8% of the administered dose in males and females, respectively.

The levels of radioactivity found in the intestinal tract, organs/tissue, blood and carcass were also comparable to those found after the administration of single doses (<0.1 - 0.4% of administered dose).

Total recovery of administered radioactivity (group means) was 99.9 and 100.2% and females, respectively.

Metabolite characterization:

Single oral administration:

Urine:

Parent compound was not identified in urine by TLC in either sex in any experiment. Seven metabolites were detected in urine, five of which were characterized. Quantitation of these metabolites (coded U_1 through U_7) in the urine of high dose males and females is shown on Appended pages 16 and 17; the metabolic pattern was comparable between dosage levels. The urinary metabolites were characterized and quantitated in urine from high dose males and females as indicated below.

Characterization of urinary metabolites in high dose males and females

Metabolite	Characterization	Percent of urinary radioactivity Males	Females
U_1	unknown	1.5	1.3
U_2	TLC, HPLC, glucuronidase = glucuronide conjugate of para-nitrophenol	5.7	8.7
U_3	TLC & HPLC = P-O-desmethyl-paraoxon-methyl	3.1	3.8
U_4	unknown	5.9	4.1
U_5	TLC & HPLC = P-O-desmethyl-parathion-methyl	5.3	6.2
U_6	TLC, HPLC & arylsulphatase = sulphate conjugate of para-nitrophenol	75.6	74.0
U_7	TLC & HPLC = para-nitrophenol	2.9	1.9

Feces:

Data which show balances of radioactivity after extraction of feces are tabulated on Appended page 18. The radioactivity in the extract was 56.1 and 47.8% of the radioactivity found in the feces of male rats exposed to the low and high doses, respectively, which corresponded to 2.1 and 1.5% of the radioactivity administered. For females, the radioactivity in the extract was 59.0 and 49.1% of the radioactivity present in feces from low and high doses, respectively, which corresponded to 1.7 and 4.4% of the radioactivity administered. No parent compound was detected in any of the extractable radioactivity.

Three metabolites (designated F_1, F_2 and F_3) were detected in the fecal extract of high dose males ranging from 0.3 to 0.8% of administered radioactivity (Appended page 19). Of these, only F_2 was characterized; TLC data indicated that this metabolite was P-O-desmethyl-paraoxon-methyl. It represented 52.7% of the extractable radioactivity.

Five metabolites (designated F_1 and F_3 through F_6) were detected in the fecal extract of high dose females ranging from 0.3 to 2.1% of administered radioactivity (Appended page 20). The F_4 metabolite was the only one of these that was characterized; TLC data provided evidence that it represented P-O-desmethyl-parathion-methyl. It represented 6.1% of the extractable radioactivity.

The investigator indicated that the lower number of metabolites detected in the urine of males may have been due to the low amount of radioactivity and

the high amount of impurities in the extracts.

Repeated oral administration:

Characterization and quantitative data were comparable to corresponding data generated with animals receiving single oral doses of ^{14}C-labeled methyl parathion these data are shown on appended pages 21, 22, 23, 24.

Summary:

Methyl parathion (U-^{14}C-phenyl-labeled) was administered orally to male and female rats in order to follow the absorption, distribution, excretion and metabolism of the radio-labeled molecule after single oral administration of the test material at 2 dose levels and after repeated oral (14x) pre-treatment using the non-radioactive test material followed by single oral administration of the ^{14}C-labeled material.

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