004465

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

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Glyphosate Registration Standard

TO: Robert Taylor (25)
Herbicide-Fungicide Branch
Registration Division (TS-767)

FROM: D. Stephen Saunders Jr., Ph.D. D. Stephen Saunders Jr. 6/19/85
Toxicologist, Section V
TOX/HED (TS-769)

THRU: Theodore M. Farber, Ph.D. 6/19/85
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Hazard Evaluation Division (TS-769) 6-19-85

Attached is the Toxicology Chapter for the Glyphosate Registration Standard. Included are the following:

1. Review of toxicity data for Glyphosate with bibliography.

2. Updated TOX "one-liners".

3. Tolerance Assessment.

The reviewer was unable to complete "Phase II Data Tables", as they were apparently not provided by the Product Manager with the use patterns identified (per memo of 5/22/84 from A. Barton and R. Brown).

Acute Toxicity

Acute oral and dermal toxicity data (Birch, 1970, MRID #00067039) place technical glyphosate in Toxicity Category III. Primary eye and skin irritation data (Birch, 1970, MRID #00067039) indicate that technical glyphosate is not a primary skin irritant (Toxicity Category IV), and is only minimally irritating to the eye (Toxicity Category III). An acute inhalation study for the technical grade of active ingredient (TGAI) has not been submitted and is required.

Chronic/Oncogenicity

Glyphosate was shown to be weakly oncogenic in the mouse (Hogan, 1983), causing a slight increase in the incidence of renal tubular adenomas (a benign tumor of the kidney) in males at the highest dose tested of 30,000 ppm. These data were reviewed by the Toxicology Branch Oncogenicity Peer Review Committee which concluded that the study demonstrated an oncogenic response. A Q* has been tentatively established as 5.9×10^{-5}. The slides have been re-examined by a consulting pathologist, and data were recently submitted indicating that an additional kidney tumor had been found in control males (none were found in controls in the original examination) which would alter the Q* and statistical significance of the data. A final determination of the relevance of these data to the Agency's regulatory position for glyphosate has not been made. Other non-neoplastic changes noted in high dose males included centrilobular hypertrophy and necrosis of hepatocytes, chronic interstitial nephritis, and proximal tubule epithelial cell basophilia and hypertrophy in females. The NOEL for non-neoplastic chronic effects was the mid dose, 5,000 ppm. This study is classified as Core-Minimum data.

The lifetime feeding study in rats (Lankas and Hogan, 1981, MRID 00093879) tested dietary concentrations of glyphosate of 0, 30, 100, and 300 ppm. These concentrations were calculated to be equivalent to doses of 0, 3, 10, and 31 mg/kg/day in males and 0, 3, 11, and 34 mg/kg/day in female rats. Thus, the doses tested in the rat chronic study were about 1/100 of those tested in the mouse study. Although no effect of treatment on the incidence of non-neoplastic lesions was noted, a marginal increase in the incidence of interstitial cell tumors of the testes was observed. It was concluded that the observed increase, although slightly higher in incidence than historical controls, was insufficiently large to demonstrate an oncogenic potential. An independent review of the data raised a question of possible thyroid carcinoma in high dose females. After a review of the slides by a consulting pathologist, the Agency concluded that the data did not demonstrate a carcinogenic response in the thyroid.

However, in view of the large difference in doses between the rat and mouse studies, the Oncogenicity Peer Review Committee speculated that "a toxic, or MTD [Maximally Tolerated Dose] was not reached in [the rat] study", and that at doses "close to an

MTD, tumors might have been induced". The rat study was re-reviewed for evidence that the highest dose tested was an MTD. No effect of treatment on survival, body weight gain, clinical pathology, or findings at necropsy was noted. Therefore, there is no evidence that the highest dose tested was an MTD. A rationale for the selection of doses in the study is required. If an acceptable justification cannot be provided, a repeat study with an MTD is required. This study is now tentatively re-classified as Core-Supplementary data.

A chronic feeding study in a non-rodent species (i.e. dog) has not been submitted and is required.

Subchronic Toxicity Studies

No acceptable rat or dog subchronic feeding studies are available for technical glyphosate. IBT studies had been submitted for both species, however were found to be invalid.

A 3-month subchronic study in mice (Street et al., 1980, MRID #00036803) tested dietary concentrations of 0, 5000, 10000, and 50000 ppm of technical glyphosate. A decrease in body weight gain was noted in high dose mice, however no gross or microscopic changes were observed at necropsy. The study was classified as Core-Supplementary data because hematology, clinical chemistry, and urinalysis measurements were not performed.

Teratology and Reproduction

Acceptable rabbit (Rodwell et al., 1980, MRID #00046363) and rat (Rodwell et al., 1980a, MRID #00046362) teratology studies have been submitted. No evidence of teratogenicity was observed in either study. In the rat study, evidence of fetotoxicity in the form of unossified sternebrae was noted in fetuses from high dose (3500 mg/kg/day) dams. This dose was also toxic to dams as evidenced by weight gain deficits, altered physical appearance, and mortality during treatment. The NOEL for fetal and maternal toxicity was 1000 mg/kg/day, and the study was classified as Core-Minimum data.

In the rabbit study, the highest dose tested (350 mg/kg/day) was toxic to does as evidenced by altered physical appearance and mortality. In spite of the toxicity of the high dose, no treatment-related fetal effects were noted. The NOEL for maternal toxicity was 175 mg/kg/day, and the study was classified as Core-Minimum data.

In the three-generation rat reproduction study (Street, 1981) and addendum (Street et al., 1982) the most significant finding was focal, unilateral, renal tubular dilation in the kidneys of male pups from the F_{3b} generation of high dose dams (30 mg/kg/day). The NOEL for this effect was 10 mg/kg/day. No effects on fertility or reproductive parameters were noted. The study was classified as Core-Minimum data.

Mutagenicity

Acceptable studies have been submitted to satisfy the Agency's testing requirements for gene mutations, chromosomal aberrations, and primary DNA damage. Glyphosate was negative for gene mutations in chinese hamster ovary cells (Li et al., 1983) in the presence or absence of microsomal activation. Glyphosate was also negative for gene mutations in bacteria, with or without activation (Inst. of Env. Tox. [Tokyo], 1978; Monsanto Env. Health Labs. #LF-78-161, 1978). Glyphosate was negative for chromosomal aberrations in the mouse dominant lethal test (Rodwell et al., 1980b, MRID #00046364), and in the in vivo cytogenetics assay (Li, 1983; Ridley, 1983). No primary DNA effects were seen with glyphosate in the B. subtilis rec assay (Inst. of Env. Tox. [Tokyo], 1978) or in the rat hepatocyte DNA repair assay (Williams, 1983).

Neurotoxicity

Even though glyphosate is not a typical organophosphate, a delayed neurotoxicity study was conducted in chickens at Industrial Bio-Test Laboratories (Fletcher and Arceo, 1976, MRID #00054494). Although no evidence of neurotoxicity was noted in the study, the validation report for this study noted an absence of raw data for dose preparation and administration, body weight measurements, and pathological observations for untreated and positive control birds. After evaluation of the study for scientific content, the study was classified as invalid on the basis of the extensive gaps in the raw data supporting study findings and conclusions.

Since glyphosate is not an organophosphate insecticide, a repeat study is not required.

Metabolism

Available metabolism data (Colvin et al., 1973; Colvin et al., 1973a) demonstrate that glyphosate is rapidly excreted by rats, as >90% of the administered dose was eliminated within 48 hours of treatment. In males, the majority of excretion was via the feces (80%), and about 15% of the administered dose was eliminated in the urine. In females, about 40% of the administered dose was excreted in the urine, which suggests that female rats absorbed more glyphosate from the gastrointestinal tract than did males.

After a single oral or intraperitoneal dose less than 1% of the administered dose was retained at 120 hours after treatment. In animals fed 1, 10 or 100 ppm of ^{14}C-glyphosate for 14 days, a steady-state equilibrium between intake and excretion of label was reached within about 8 days. The amount of radioactivity excreted in the urine declined rapidly after withdrawal of treatment. By 10 days after withdrawal, detectable levels of radioactivity were measured in the urine and feces of only the rats fed 10 or 100 ppm of the test diet. Only minimal residues of 0.1 ppm or less remained in the tissues of high dose rats after 10 days

of withdrawal, with no single tissue showing a significant difference in the amount of label retained.

The submitted studies are deficient in that data for the analysis of excreta for the presence of metabolites were not submitted, and only 1-3 animals were used in each experimental group. The submitted data demonstrated differential effects on excretion and retention of radioactivity depending on the molecular location of the radioactive label. These findings are strong evidence that some metabolism of glyphosate occurred in rats.

The metabolism studies for glyphosate are classified as Core-Supplementary data, and repeat studies are required.

N-Nitroso-Glyphosate

Residue Chemistry Branch (RCB) has determined that technical glyphosate contains N-nitroso-glyphosate (NNG) as a contaminant at levels of 0.1 ppm or less. Current policy on nitroso contaminants is that oncogenicity testing for these contaminants will normally be considered only in those cases in which the level of nitroso compounds exceeds 1.0 ppm. Therefore, although a chronic feeding study in rats was reviewed and found unacceptable, no additional studies are requested at this time.

Acute oral toxicity data for NNG (Younger Labs., 1975; ibid, 1976) place it in Toxicity Category III. Other acute toxicity data for NNG are not available in Toxicology Branch files.

Chronic toxicity studies in the dog and rat were conducted at IBT. After a raw data audit, both studies were judged to be Supplementary data. Both studies were then evaluated for scientific acceptability, and the rat study (Morrow et al., 1979) was classified as Core-Invalid due to dosing of the control groups with an excessive amount of NaCl which resulted in high mortality of control animals. The dog study (Jenkins et al., 1979) remained Core-Supplementary after scientific evaluation due to the lack of supporting raw data as identified in the raw data audit validation report. The only apparent treatment-related findings in the dog study were an increase in absolute and relative kidney weights and in blood glucose in high dose (30 mg/kg/day) females. The NOEL for this apparent effect was 10 mg/kg/day.

A 90-day subchronic oral toxicity study with NNG was conducted in the rat (Pharmacopathics Research Labs., 1982). The principal effect of treatment was a dose-related decrease in survival, food consumption and body weight gain. A NOEL was not established in this study since these effects were noted at the lowest dose tested, 3000 mg/kg/day. The study was classified as Supplementary data due to inadequate reporting of clinical sign and necropsy data, and inadequate identification of the test material.

A rat metabolism study conducted with NNG (Sutherland, 1978) demonstrated that NNG is rapidly absorbed and excreted, with the kidneys the preferential route of elimination. These findings are in direct contrast with the results of the metabolism studies with glyphosate, which found that absorption from the gut was poor and the majority of excretion occurred in the feces due to unabsorbed radiolabel. Tissue residues after 5 consecutive doses were minimal, as no tissue contained more than 1.5 ppm of radiolabel.

No acceptable studies for mutagenic or reproductive effects are available at present for NNG.

Plant Metabolite- Aminomethylphosphonic Acid

Residue Chemistry Branch has determined that the metabolite aminomethylphosphonic acid (AMPA) is formed on plants in amounts that can range as high as 28% of the total residue on the plant. Since the extent of glyphosate metabolism was not adequately addressed in the rat metabolism study, the possibility exists that the AMPA metabolite could pose a hazard to humans that was not evaluated by testing the parent compound, glyphosate. If an acceptable rat metabolism study is submitted which demonstrates significant conversion in animals of glyphosate to AMPA, additional studies on this metabolite may be unnecessary.

Acute oral toxicity and primary skin irritation data place AMPA in toxicity category IV (Birch, 1973, MRID 00084125). The primary eye irritation study demonstrated that AMPA was slightly irritating to the eye, corresponding to toxicity category III (ibid).

A 90-day subchronic feeding study was submitted (Street, et al., 1979) that demonstrated irritation of the urinary bladder in rats treated with 1200 mg/kg/day, the LEL in this study. This irritation was manifested in the form of hyperplasia of the cells lining the bladder, and was noted with increased incidence and severity at the highest dose tested, 4800 mg/kg/day. Epithelial hyperplasia of the renal pelvis was also noted in high dose rats. The NOEL for this effect was 400 mg/kg/day, and the study was classified as Core-Minimum data.

A rat metabolism study (Colvin et al., 1973b) demonstrated that AMPA is rapidly excreted as the parent compound. No evidence for bioaccumulation was noted in this study, which was classified as Supplementary data because the number of animals studied was not reported, only males were studied, and the effects of a minimally toxic dose and repeated non-toxic doses on excretion, metabolism, and accumulation were not assessed.

The limited data available for AMPA do not suggest that this compound poses any hazard distinct from that of the parent compound. No studies are available by which to assess potential mutagenic, reproductive, oncogenic, or chronic effects of AMPA. The need for additional testing of this compound will be assessed after the submission of an acceptable rat metabolism study with glyphosate.

Tolerance Assessment

The Acceptable Daily Intake (ADI) for glyphosate is currently based on the finding of renal tubular dilatation in F_{3b} pups in the rat three generation reproduction study. The NOEL for this effect was 10 mg/kg/day. Using a 100-fold safety factor, the ADI for glyphosate is therefore 0.1 mg/kg/day which is equivalent to a Maximum Permissible Intake of 6.0 mg/day in a 60 kg individual. Existing tolerances produce a Theoretical Maximum Residue Contribution (TMRC) of 1.4238 mg/day from a 1.5 kg diet, which occupies

$$\frac{1.4238 \text{ mg/day}}{6.0 \text{ mg/day}} \times 100 = 23.73\% \text{ of the ADI.}$$

With the submission of the mouse oncogenicity study with apparent findings of benign kidney tumors in males, a preliminary Q* was calculated by Dr. H. Lacayo, Tox. Branch Statistician, to be 5.9×10^{-5} by a multihit model. Using the expression:

$$\text{Risk} = \text{Exposure} \times Q^*$$

the risk associated with existing tolerances is:

$$\text{Risk} = E \times Q^* = \frac{(1.4238 \text{ mg/day})}{60 \text{ kg}} \times (5.9 \times 10^{-5})$$

$$= (2.37 \times 10^{-2}) \times (5.9 \times 10^{-5}) = \underline{1.40 \times 10^{-6}}.$$

It should be noted that a final determination of the relevance of the mouse oncogenicity data to the Agency's regulatory position has not yet been made.

004465

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Acceptable Daily Intake

Wt,other Hum mg/kg	Hum %	S.F.	DI mg/kg/day	ADI mg/day(60kg)
10.00	200.00	100	0.1000	6.0000

Published Tolerances

Crop	Tolerance	Food Factor	mg/day(1.5kg)
Grain Crops(84)	0.100	13.75	0.02063
Avocados(9)	0.200	0.03	0.00005
Citrus Fruits(33)	0.200	3.41	0.01149
Coffee(36)	1.000	0.75	0.01113
Grapes, inc raisins(69)	0.100	0.49	0.00074
Leafy Vegetables(80)	0.200	2.76	0.00528
Nuts(101)	0.200	0.10	0.00031
Pome Fruits(120)	0.200	2.79	0.00537
Root Crop Veg(130)	0.200	11.00	0.03299
Seed&Pod Veg(143)	0.200	3.00	0.01026
Palm Oil(202)	0.100	0.03	0.00005
Pistachio nuts(210)	0.200	0.03	0.00005
Asparagus(5)	0.200	0.14	0.00043
Pineapples(7)	0.200	1.42	0.00426
Olives(104)	0.100	0.06	0.00005
Stone Fruits(151)	0.200	1.25	0.00374
Sugar,canebeet(154)	2.000	3.04	0.10915
Molasses(50)	20.000	0.03	0.00920
Cranberries(44)	0.200	0.03	0.00005
Cottonseed (oil)(41)	15.000	0.15	0.03375
Kidney(203)	0.500	0.03	0.00023
Liver(211)	0.500	0.03	0.00023
Peanuts(115)	0.100	0.36	0.00054
Guava(164)	0.200	0.03	0.00009
Papayas(169)	0.200	0.03	0.00009
Mangoes(66)	0.200	0.03	0.00009
Soybeans (oil)(146)	0.000	0.92	0.03263
Pineapple(123)	0.100	0.30	0.00044
Fish,shellfish(59)	0.250	1.06	0.00466
Cucurbits(49)	0.100	2.84	0.00426
Fruiting Vegetables(60)	0.100	2.99	0.00449
Small Fruit,berries(146)	0.100	0.83	0.00124
Hops(73)	0.100	0.03	0.00005
Potable Water(198)	0.500	133.33	1.00000
Tea(162)	4.000	0.07	0.00429

RfD	TMRC	% ADI
6.0000 mg/day(60kg)	1.3660 mg/day(1.5kg)	22.8

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Unpublished,Tox Approved 2F2660,2G2660

Crop	Tolerance	Food Factor	mg/day(1.5kg)
Soybeans (oil)(148)	4.000	0.92	0.03509
Coconut(35)	0.100	0.03	0.00005

9

MPI	TMRC	% ADI
6.0000 mg/day(60kg)	1.4236 mg/day(1.5kg)	23.73

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Current Action 5F3170

CROP	Tolerance	Food Factor	mg/day(1.5kg)
Sugar,cane&beet(154)	0.000	3.64	0.00000
Kidney(203)	0.000	0.03	0.00000
Liver(211)	0.000	0.03	0.00000

MPI	TMRC	% ADI
6.0000 mg/day(60kg)	1.4236 mg/day(1.5kg)	23.73

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Younger Labs. (1976, Author unknown) Study No. Y-76-122; Accession #229785 MRID

Tox Chem No. 661 A Glyphosate

File Last Updated 8/22/84

Current Date 6/10/85

Study/Lab/Study #/Date	Material	EPA Accession No.	Results/LD50, LC50, PIS, NOEL, LEL	TOX Category	CORE Grade/ Doc. No.
Teratology - rabbit; IBT; #J-568, BTL 71-36; 6/30/72	TECH	009856	IBT Invalid		000266 003853
Teratology - rat; IRDC; #401-054; 3/21/80	TECH 98.7%	242516	Negative for teratogenic effects. Maternal NOEL = 1000 mg/kg/day Maternal LEL = 3500 mg/kg/day (inactivity, death, stomach hemorrhages, reduced body weight gain) Fetotoxic NOEL = 1000 mg/kg/day Fetotoxic LEL = 3500 mg/kg/day (unossified sternebrae) Levels tested: 0, 300, 1000 & 3500 mg/kg/day		Minimum 000119
Teratology - rabbit; IRDC; #401-056; 2/29/80	TECH 98.7%	242516	Negative for teratogenic effects. Fetotoxic NOEL=350/mg/kg/day Maternal NOEL=175 mg/kg/day Maternal LEL=350 mg/kg/day (death, soft stools, diarrhea, nasal discharge) Levels tested: 0, 75, 175 & 350 mg/kg/day		Minimum 000120 000119
Teratology - rabbit; IBT; #561-05275	Technical		IBT-Invalid		000270
3 Generation reproduction - rat; IBT; #B-566, BTL 71-34; 7/26/73	TECH		IBT - Invalid per Canadian revalidation of 4-8-81.		Invalid 000276 000280 002193

Tox Chem No. 661 A - Glyphosate

Study/Lab/Study #/Date	Material	EPA Accession No.	Results/LD50, LC50, PIS, NOEL, LEL	TOX Category	CORE Grade/ Doc. No.
3 Generation reproduction - rat; Biodynamics; #77-20663; 3/31/81 & 7/6/82	Glyphosate Lot # XHJ-64	245909 247793	Systemic NOEL =10 mg/kg/day based on pathological findings of renal focal tubular dilation in male F3b weanling at high level (30 mg/kg/day)		Supplementary 001062 Upgraded to Minimum 002124
21 Day oral - rabbit; IBT; 601-05044; BTL 74-7	Technical		IBT-invalid		000279
21 Day dermal - rabbit; IRDC;#401-168; 3/10/82	Glyphosate Technical	247228	NOEL = 1000 mg/kg/day LEL = 5000 mg/kg/day (Slight erythema and edema) Levels tested: 0, 100, 1000 & 5000 mg/kg/day		Minimum 001814
90-Day oral - rat; IBT; #B1020, BTL 71-57; 6/26/72	CP 67573 (N-phosphoro methyl glycine)		IBT Invalid		002179 003853
90 Day feeding - dog; IBT; #C-1021, BTL 71-58; 6/19/72	TECH	009856	IBT Invalid		000263 002177
90-Day feeding - mice; Biodynamics; #77-211; 12/31/79	Technical	242799	NOEL = 10,000 ppm LEL = 50,000 (reduced body weight gain)		Supplementary 000254
90-Day oral- rat; IBT; BTL 7157,B 1020; 6/6/72	CP 67573 Technical		Invalid (evaluation date 9/14/72)		002179
18 Month oncogenic - mice; IBT;B-569, BTL 71-37; 9/19/73	Technical		IBT-invalid		000277

004465

Study/Lab/Study #/Date	Material	EPA Accession No.	Results/LD50, LC50, PIS, NOEL, LEL	TOX Category	CORE Grade/ Doc. No.
2 Year feeding/oncogenic - rat; IBT; B-564, BTL 71-32; 1/14/74	Technical	112789	IBT-invalid		000265 000262 000280
2-Year feeding - dog; IBT;#J-565 (651-00565), BTL 71-33; 11/30/73	Technical (CP 67573 Acid form)	112789 94161	Evaluation considering Canadian validation findings of 6/19/78 and additional data submitted by Monsanto on 7/2/82 and reclassified as IBT invalid		000265 000269 002134
26 month feeding - rat; Bio/dynamic; #77-2062; 9/18/81	Technical 98.7% a.i.	246617 to 246621	Oncogenic NOEL > 31 mg/kg/day (HDT) Sys NOEL > 31 mg/kg/day (HDT) Supplementary due to no evidence that an MTD was used in this study.		Minimum 001425 002175 002666 Supplementary 004465
Neurotoxicity - hen;IBT; 8580-09117; 12/22/76	Technical		IBT valid per Canadian validation. Invalid per scientific evaluation.		000279 Invalid 004465
Mutagenic - gene mutation, CHO/HGPRT; Monsanto; #ML-83-155; 10/20/83	Technical	251737	Not mutagenic with or without S-9 activation		Acceptable 003868
Mutagenic - DNA repair (Rat hepatocytes); Monsanto; AH-83-181; 10/21/83	Glyphosate Technical	251737	Negative for DNA damage at concentrations between 1.25×10^{-5} and 1.25×10^{-1} mg/ml.		Acceptable 003868
Mutagenic - *in vivo* bone marrow cytogenetic; Monsanto; #ML-83-236; 10/20/83	Glyphosate Technical	251737	Negative at 1000 mg/kg		Unacceptable 003868 Acceptable 004348

Tox Chem No. 661A

Study/Lab/Study #/Date	Material	EPA Accession No.	Results/LD50, LC50, PIS, NOEL, LEL	TOX Category	CORE Grade/ Doc. No.
Mutagenic - Monsanto; #ML-83-60; 10/21/83			Range finding for in vivo cytogenetics study. No effect on cell viability or mitotic index at doses of 200-1000 mg/kg i.p.		Acceptable for range finding 003868
Pharmacokinetics; Monsanto; #830109; 10/23/83	Glyphosate Technical	251737	T 1/2 of 7.6 hours (males) T 1/2 of 4.2 hours (females)		Acceptable 003868
Mutagenic - mice; IBT; #E-567; STL 71-35; 1/24/72	TECH	009856 234134	IBT Invalid		003853 000271
Mutagenic - dominant lethal - mice; IRDC; #401-064; 4/16/80	TECH 98.7%	242516	Negative up to 2000 mg/kg Levels tested: 0, 200, 800 and 2000 mg/kg.		Minimum 000120
Mutagenic - host mediated - rat & mice; IBT; #623-07508			Negative at up to 100 ug/plate(HDT) IBT Invalid per Dynamac validation report 12/27/83; Contract Number 68-01-6561.		000275 000270 000275
Mutagenic - microbial; Institute of Environmental Toxicology (Tokyo); 7/20/78	Technical 98.4% a.i.		(1) Rec-assay (with B. subtilis) negative at 2,000 ug/disk (2) Reverse mutation with and without liver metabolic activation system (with S. typhimurium) negative		Minimum 000258
Mutagenic - microbial; Monsanto Environmental Health Labs.; LF-78-161 6/16/78	Technical 98.4% a.i.		Negative for gene mutations in Salmonella up to 1000 ug/plate with or without metabolic activation.		Minimum 000258

Tox Chem No. 661A

Study/Lab/Study #/Date	Material	EPA Accession No.	Results: LD_{50}, LC_{50}, PIS, NOEL, LEL	TOX Category	CORE Grade/ Doc. No.
Metabolism - rat; Monsanto Ag. Res. Dept.; Report #297; 6/15/7; and Report #309; 10/73	^{14}C-glyphosate 95-98% a.i.		90-100% clearance of single oral or i.p. dose after 120 hours. Females appeared to absorb about 3x as much as males. Levels tested: 0, 1, 10, 100 ppm in diet for 14 consecutive days. Only minimal residues of 0.1 ppm or less retained in tissues after 10 day withdrawal period. Supplementary because purity, activity of label used in repeated dose study not given; only 1-3 animals per group; data for metabolites in excreta not provided.		Supplementary 004465
Cholinesterase inhibition - rat; IBT; 601-06527, BTL 75-3; 3/7/73	Technical		IBT-invalid		000279 000270
Acute oral LD_{50} - rat; Younger Labs; #Y-70-90; 9/18/70	TECH	009856	LD_{50} (M&F) = 4320 (3930-4750)mg/kg	III	003853
Acute oral LD_{50} - rabbit; IBT; A-2277, BTL 72-109; 12/6/72	Technical administered as a 35% w/v suspension in methylcellulose	112791	LD_{50} = 3800 (2836 - 5092) mg/kg IBT - Valid	III	003853 000278 000264
Acute dermal LD_{50} - rabbit; Younger Labs; #Y-70-90; 9/18/70	TECH	009856	LD_{50} (F) $\geq$ 7940 mg/kg LD_{50} (M) $\geq$ 5010 mg/kg	III	003853

Tox Chem No. 661A

Study/Lab/Study #/Date	Material	EPA Accession No.	Results: LD_{50}, LC_{50}, PIS, NOEL, LEL	TOX Category	CORE Grade/ Doc. No.
Acute inhalation LC_{50} - rat; IBT; #663-07290, BTL 74-116A; 5/20/75	Technical		IBT-invalid		000279
Primary dermal irritation - rabbit; Younger Labs; #Y-70-90; 9/18/70	TECH	009856	P.I.S.= 0.0/8 (24 hour exposure)	IV	003853
Primary eye irritation-rabbit; Younger Labs; #Y-70-90; 9/18/70	TECH	009856	P.I.S. = 12.6/110 at 1 hour		Minimum 000265 000278
Acute intraperitoneal LD_{50} - rat; Younger Lab; #Y-73-112; 6/20/73	Technical (10% in corn oil)		LD_{50} = 470 (410-540) mg/kg		000279
Acute subcutaneous LD_{50}- rat; Younger Labs; #Y-75-151; 6/23/75	Technical 10% and 20% in corn oil		LD_{50} > 5,010 mg/kg (highest level tested) Reduced appetite and activity (No Deaths)		000279
Dissimilation chemicals metabolte or impurity or contaminant or salt or photodegradent or etc			Caswell No. 37 C (Aminomethyl phosphonic acid) Caswell No. 509 E (Isopropylamino salt of glyphosate) Caswell No. 788 A (Sodium glyphosate) Caswell No. 604 I (N-Nitrosoglyphosate-Na salt)		
Risk assessment; OPP/HED/TOX					002860

004465

Tox Chem No. N-Nitrosoglyphosate File Last Updated 8/22/84 Current Date 6/13/85

004465

Study/Lab/Study #/Date	Material	EPA Accession No.	Results: LD_{50}, LC_{50}, PIS, NOEL, LEL	TOX Category	CORE Grade/ Doc. No.
90 day oral - rat Pharmacopathics Research Labs.; #7934; 6/25/82	CP 76100 N-nitroso-glyphosate % a.i. not given.	249157 249158	Levels tested: 0, 3000, 6000 mg/kg by gavage NOEL = Not established. LEL = 3000 mg/kg/day (Decreased survival, food consumption, and body weight.		Supplementary
13 month oral - hamster; Bio/dynamics Inc.; #76-1401; 6/29/79	CP 76100 N-nitroso-glyphosate 19.8% a.i.	249161 249162 249163	Levels tested: 0, 3, 10, 30 mg/kg by gavage. Invalid due to excessive mortality in all test groups.		Invalid
2 year oral - rat IBT; #8560-08924; 5/14/79	CP 76100 N-nitroso-glyphosate 19.8% a.i.	247745 to 247752	Levels tested: 0, 3, 10, 30 mg/kg by gavage. Supplementary per Dynamac raw data validation: Contract #68-01-6561 Invalid per scientific evaluation due to inappropriate treatment of control group.		Invalid 004465
2 year oral - dog IBT; #8580-08922; 5/8/79	CP 76100 N-nitroso-glyphosate 19.8% a.i.	247753	Levels tested: 0, 3, 10, 30 mg/kg by capsule. Supplementary per Dynamac raw data validation: Contract #68-01-6561 Evaluation: NOEL = 10 mg/kg/day LEL = 30 mg/kg/day (Increased absolute and relative kidney weights, increased blood glucose in females). Core-Supplementary per raw data validation.		Supplementary 004465
Teratology - rabbit; IBT; #8580-08921; 1/4/77	CP76100 N-Nitroso glyphosate	229785	IBT - Invalid		000275

Tox Chem No. 604I N-Nitrosoglyphosate

File Last Updated 8/22/84

Current Date 6/13/85

004465

Study/Lab/Study #/Date	Material	EPA Accession No.	Results: LD_{50}, LC_{50}, PIS, NOEL, LEL	TOX Category	CORE Grade/ Doc. No.
Mutagenic, Ames - Salmonella and Saccharomyces; Litton Bionetics; #2547; 6/22/76	CP76100 N-nitroso glyphosate	229785	Negative with or without activation at concentrations of up to 5 ul/plate		000275 Unacceptable 000271
Mutagenic, dominant lethal; IBT; 8533-08929; 1/4/77	CP76100 N-nitroso glyphosate	229785	IBT - Invalid Dynamac Corporation - Contract No. 68-01-6561. Accepted by EPA 5/14/82		000275 002970 000271
Acute oral LD_{50} - rat; Younger Labs.; Y-76-122; 4/19/76	CP 76100 N-nitroso glyphosate, 20% aqueous solution	229785	LD_{50} (combined) = 7.6 (7.07-8.21) g/kg (in terms of active ingredient)	IV	000275
Acute oral LD_{50} - rat; Younger Labs.; Y-75-309; 12/12/75	CP 76100 N-nitroso glyphosate, 20% aqueous solution	229785	LD_{50} (combined) = 4.35 (4.09-4.61) g/kg (in terms of active ingredient)	III	000275
Metabolism - rat; Monsanto Ag. Research; MSL 0242; 4/26/78	$^{13}C/^{14}C$-N-nitroso-glyphosate 90:10 ratio	233913	Rapidly absorbed from GI, 90% of 1 mg/rat oral dose eliminated in the urine in the first 24 hours; 60% eliminated in urine after 5 doses of 30 mg/kg, 30% in feces no accumulation in tissues; no biotransformation of label.		Supplementary 004465
Dissimilation chemicals-metabolite or impurity or contaminant or salt or photodegradent or etc			Glyphosate #661A; Isopropylamine salt of glyphosate 471AAB; Sodium salt of N-nitroso-glyphosate 604AAB		

Tox Chem No. 37 C Aminomethyl phosphonic acid File Last Updated 7/10/84 Current Date 6/13/85

004465

Study/Lab/Study #/Date	Material	EPA Accession No.	Results: LD_{50}, LC_{50}, PIS, NOEL, LEL	TOX Category	CORE Grade/ Doc. No.
90 Day feeding - rat; IRDC; #401-050	Aminomethyl phosphonic acid	241351	NOEL = 400 mg/kg/day LEL = 1200 mg/kg/day (weight loss, histopathologic lesions in urinary bladder) Levels tested: 0, 400, 1200, 4800 mg/kg/day		Minimum 000256
Metabolism - rat; Monsanto Agricultural Division Research Dept.; Report #303; 8/73	^{14}C-Aminomethyl phosphonic acid		93% of oral dose excreted unchanged by 72 hours, 20% excreted in urine. 0.06% retained in tissues. Only males studied, number of animals not stated, no repeated dose or toxic dose studies conducted.		Supplementary 004465
Acute oral LD_{50} - rat; Younger Labs; Y-73-19; 3/7/73	Aminomethyl phosphonic acid; 40% solution in corn oil	094161	LD_{50} (M & F) = 8300 (7300 - 9460) mg/kg	IV	000278 000273 003160
Primary eye irritation - rabbit; Younger Labs; Y-73-19; 3/7/73	Aminomethyl phosphonic acid	094161	Maximal score 10.0/110 at 1 hr. Slight irritant	III	000273 000278
Primary dermal irritation - rabbit; Younger Labs; Y-73-19; 3/7/73	Aminomethyl phosphonic acid	094161	PIS = 0.0/8.0 No irritation	IV	000273 000278
Metabolite (plant) of			glyphosate (Caswell #661A)		

Data Evaluation Record

Chemical: Glyphosate (ROUNDUP® Technical); 98.7% a.i.

Study Identification: "A Lifetime Feeding Study of Glyphosate in Rats";

EPA ID No.:	524-308
Accession No.:	246617,18,19,20,21
Project No.:	77-2062 (Bio/dynamics)
Report date:	12/23/81
Submitted:	1/20/82
Sponsor:	Monsanto Agricultural Products Co. St. Louis, MO. 63166
Test facility:	Bio/dynamics, Inc. East Millstone, N.J. 08873
Study authors:	G.R. Lankas and G.K. Hogan.

Reviewed by: D. Stephen Saunders Jr., Ph.D.
Toxicologist, Section V
TOX/HED (TS-769)

Approved by: Laurence D. Chitlik, DABT
Head, Section V
TOX/HED (TS-769)

Conclusions: Not an oncogen at the HDT, 31 mg/kg.
NOEL for chronic effects = 31 mg/kg.

Classification: Core-Supplementary No evidence of a Maximally Tolerated Dose (MTD).

Background

This study was previously reviewed and classified as Core-Minimum data (memo W. Dykstra to R. Taylor, 2/18/82). In that review, the NOEL for chronic effects was established at 3.0 mg/kg/day based on lymphocytic hyperplasia of the thymus in female rats, and no oncogenic potential was demonstrated at the highest dose tested (HDT) of 31-34 mg/kg/day. The Registrant responded with a letter from Dr. M.G. Robl of Environmental Pathology Labs., Inc., who concluded that the observed incidence of lymphocytic hyperplasia of the thymus was "of no biological significance in this study", and therefore not related to treatment with the test article. After reviewing the submitted information, the Agency agreed with the Registrant, and the NOEL was then revised to the HDT, 31 mg/kg/day (memo W. Dykstra to R. Taylor, 4-8-82).

Subsequently, a mouse oncogenicity study was submitted and reviewed (memo Dykstra to Taylor, 4/3/85). In that study, much higher doses of 1,000, 5,000, and 30,000 ppm in the feed were administered to the mice, in contrast to the doses in the rat study of 30, 100, and 300 ppm. Thus, the doses tested in the rat study were only 1/100 of those used in the mouse study. In addition, a positive oncogenic response was demonstrated in the mouse study in the form of kidney adenomas at the HDT. It was concluded by the Toxicology Branch Glyphosate Peer Review Panel (memo to R. Taylor, 3-4-85) that "a toxic, or MTD, level was not reached in this study....at toxic levels at or close to a MTD, tumors might have been induced".

The purpose of the present review is to re-assess the submitted rat chronic feeding study for evidence of toxic effects at the HDT.

Materials and Methods

A. Materials: 1) Test material- Glyphosate, 98.7% a.i.

2) Doses tested- 0, 30, 100, and 300 ppm in feed.

3) Test animal- Sprague-Dawley (CD) rats, obtained from Charles River Labs., Wilmington, Mass.; 50/sex/dose.

B. Methods: Previously reviewed (memo W. Dykstra to R. Taylor, 2-18-82). No significant deviations from Agency guidelines were noted that would compromise the study.

Results

A. Mortality and Clinical Signs- Data for clinical signs were not submitted. It was stated in the report narrative that the observed clinical signs of alopecia, lacrimation, nasal discharge, and rales were "present in animals in all groups....in approximately the same incidence and are common observations in the laboratory rat.... Therefore, it is concluded that the administration of the test substance did not significantly affect the physical condition of the animals on test in this study."

Mortality data were submitted in the form of a month-by-month summary of mortality and as individual animal fates. No effect of treatment on survival was apparent (Table 1 of this review). It was stated in the report narrative that "there was no significant difference between the control and treated groups of both sexes with regard to the survival rate during the course of this study."

Table 1. Survival[a]

Dose (ppm)	12 Months Males	12 Months Females	18 Months Males	18 Months Females	24 Months Males	24 Months Females	26 Months[b] Males	26 Months[b] Females
0	47/50[c] (94%)[d]	50/50 (100%)	42/50 (84%)	47/50 (94%)	22/50 (44%)	26/50 (52%)	15/50 (30%)	18/50 (36%)
30	49/50 (98%)	49/50 (98%)	45/50 (90%)	46/50 (92%)	28/50 (56%)	35/50 (70%)	26/50 (52%)	23/50 (46%)
100	47/50 (94%)	50/50 (100%)	44/50 (88%)	47/50 (94%)	23/50 (46%)	32/50 (64%)	16/50 (32%)	30/50 (60%)
300	50/50 (100%)	48/50 (96%)	49/50 (98%)	43/50 (86%)	33/50 (66%)	24/50 (48%)	26/50 (52%)	15/50 (30%)

[a]data excerpted from submitted study.
[b]study terminated after 26 months.
[c]number alive/number placed on test
[d]percent survival, calculated by reviewer.

b. Body Weights- Body weight data were submitted as individual animal values and as summary tabulations.

No effect of treatment on body weight in males was apparent (Table 2). A slight (5%) decrease in group mean body weight compared to control was noted in high dose males for the last year of the study, however the difference was not statistically significant. The investigators stated that "no statistically significant differences were noted among the mean body weights of the treated males as compared to the Group I controls during the course of the study.... Because [the observed] effect was slight and not evident at termination of the study and did not affect survival, it is not considered to be toxicologically significant."

Similarly, no treatment-related effects were apparent in females (Table 2). Although slight decreases were noted in treated rats from about week 40 until termination, the effect was not particularly dose-dependent as the greatest decrease was often in the low dose group (30 ppm). The investigators stated that "this effect was not dose-related in the treated females and may be due to biological variation".

Table 2. Body Weights[a]

	Males				Females			
	DOSE (ppm)							
Week	0	30	100	300	0	30	100	300
0	182± 10	182± 13	183± 11	183± 12	141± 10	138± 8	139± 9	137± 9
26	547± 53	547+ 54 (100%)[b]	546+ 51 (100%)	536+ 46 (98%)	294± 32	293+ 31 (100%)	288+28 (98%)	287+ 31 (98%)
52	664± 79	655+ 75 (99%)	650+ 68 (98%)	634+ 64 (95%)	366± 57	356+ 51 (97%)	347+51 (95%)	354+ 56 (97%)
78	724±104	725+ 96 (100%)	699+ 85 (97%)	691+ 79 (95%)	427± 94	404+ 71 (95%)	406+65 (95%)	420+ 87 (98%)
104	693±101	689+ 88 (99%)	702+ 96 (101%)	691+ 89 (100%)	453±103	432+101 (95%)	438+73 (97%)	444+ 83 (98%)
T[c]	694±135	675+113 (97%)	664+113 (96%)	692+ 94 (100%)	457±127	456+ 91 (100%)	438+81 (96%)	448+101 (98%)

[a]data excerpted from submitted study. Values are mean ± std. dev., calculated by the investigators.
[b]percent of control, calculated by reviewer.
[c]T = termination, week 110 for males, 112 for females.

C. Food Consumption and Compound Intake- Food consumption data were submitted as summary tabulations and as individual animal values.

Although statistically significant differences in food consumption were noted at some of the measured intervals, no consistent effects over time or in relation to dose were noted. The investigators stated that "Occasional statis-

tically significant differences were noted in the treated animals of both sexes relative to their respective controls. However, these differences in mean food consumption values were slight and occurred sporadically unrelated to dose level. Therefore, it is concluded that the dietary administration of Glyphosate at the doses utilized in this study did not significantly affect food consumption values in either sex."

Similarly, no effect of treatment on water consumption was noted.

D. Clinical Pathology: (1) Hematology- This parameter was measured in 10 rats/sex/dose after 4, 8, 12, 18 and 24 months of treatment. Data were submitted as summary tabulations and as individual animal values.

No effect of treatment on this parameter was noted in the original review by Dr. Dykstra (memo to R. Taylor, 2-18-82). The report narrative stated that "The few statistically significant differences noted appear to be due to random variation as no consistent treatment-related pattern is evident. On the basis of this data it is concluded that the administration of the test substance did not affect the hematology parameters evaluated."

This reviewer agrees with the assessments of the original reviewer and the study authors that no treatment-related effects were demonstrated.

(2) Clinical Chemistry- This parameter was also measured in 10 rats/sex/dose after 4, 8, 12, 18 and 24 months of treatment. Data were submitted as summary tabulations and as individual animal values.

No effect of treatment on this parameter was noted in the original review by Dr. Dykstra (memo to R. Taylor, 2-18-82). The report narrative stated that "Occasional statistically significant differences were noted, but these appear due to random fluctuaton, as no treatment-related pattern emerged. Thus,...the administration of the test substance did not significantly affect any of the clinical biochemistry parameters evaluated during the course of this study."

This reviewer agrees with the assessments of the original reviewer and the study authors that no treatment-related effects were demonstrated.

(3) Urinalysis- This parameter was measured in 10 rats/sex/dose after 4, 12, 18 and 24 months of treatment. Data were submitted as individual animal values only.

No effect of treatment on this parameter was noted in the original review by Dr. Dykstra (memo to R. Taylor, 2-18-82). The report narrative stated that "No significant differences were noted in the urinalysis data when the control groups were compared to the treated groups for both sexes. Occasional values outside the normal range were found; however, these values occurred sporadically exhibiting no consistent pattern."

This reviewer agrees with the assessments of the original reviewer and the study authors that no treatment-related effects were demonstrated.

E. Necropsy Data: (1) Organ Weights- Absolute and relative organ weights were determined for all animals surviving to termination. Data were submitted as summary tabulations and as individual animal values.

No effect of treatment on organ weights was identified in the original review (memo Dykstra to Taylor, 2-18-82). The report narrative stated that "No statisically significant differences were noted in the terminal organ weights, organ/body weight ratios and organ/brain weight ratios of the treated groups as compared to their respective controls....no consistent pattern related to the administration of the test substance was evident."

This reviewer agrees with the assessments of the original reviewer and the study authors that no treatment-related effect on organ weights was demonstrated.

(2) Gross Observations- Individual animal data only were submitted, without summary tabulations of gross observations. A table which correlated gross observations with corresponding histological diagnosis was also provided.

No effect of treatment on the incidence of gross observations was identified in the original review (memo Dykstra to Taylor, 2-18-82). The report narrative stated that "Gross observations noted at necropsy were similar in incidence between control and treated animlas of both sexes. Lesions noted were those commonly found in chronic studies conducted in this laboratory on the same strain of rats."

Although a summary incidence table for gross observations was not submitted, this reviewer agrees with the assessments of the original reviewer and the study authors that no effect of treatment on gross findings was demonstrated (see E.4., "Correlation of gross observations and histopathology").

(3) Histopathology- Slides were reviewed by Experimental Pathology Laboratories, Inc. (EPL). Data were submitted as individual animal findings and as summary incidence tables for all observed lesions.

In the original review, Dr. Dykstra identified lymphocytic hyperplasia of the thymus in mid and high dose females and focal vacuolization of the liver in high dose males as possible effects of treatment with the test article. Regarding effects on the thymus, the Registrant engaged Dr. Martin Robl of EPL to respond to the Toxicology Branch review (letter to R. Taylor from Monsanto, 4-6-82). Dr. Robl stated that although a slight increase in the incidence of this lesion was noted in high dose females, the severity of the observed lesion was not affected by treatment, and other organs of the lymphoreticular system were not affected. Dr. Robl concluded that "it is my opinion that the microscopic finding of lymphocytic hyperplasia in the thymus is of no biological significance in this study".

Toxicology Branch agreed with Dr. Robl's conclusion (memo Dykstra to Taylor, 4-8-82) as to possible effects on the thymus. No mention was made of the reported increase in cytoplasmic vacuolization in high dose males. In the opinion of this reviewer, this apparent change is of no toxicological significance since other effects on the liver were not observed, and the reported increase in incidence was slight (3/50 control vs. 7/50 high dose).

An increase in the incidence of interstitial cell tumors of the testes was noted in treated males (0/50 control, 3/50 low dose, 1/50 mid dose, 6/50 high dose). The Registrant presented control data that demonstrated that the historical incidence of this neoplasm for animals sacrificed at 24-29 months of age was about 10%. The incidence for high dose animals in the present study was 12%, a slight increase from the historical control. The report narrative concluded that "this comparison suggests an incidence of this tumor in the Group I [control] males which is slightly lower (0%), and incidence in Group IV [high dose] males which is slightly higher than recent historical control data. Although an effect on the incidence of this tumor due to the administration of the test substance cannot be ruled out, the data suggest that the incidence in Groups II through IV is within the normal biological variation observed for tumors at this site in this strain of rat."

This reviewer agrees that an effect of the test substance on the incidence of interstitial cell tumors in treated animals "cannot be ruled out". Even though the observed incidence of this tumor in high dose males is similar to the highest reported historical control incidence, the 0% incidence in concurrent control males must also be considered. Had a higher dose of the test chemical been tested, a more clear-cut oncongenic response may have been demonstrated.

An independent review of this study raised a question of possible thyroid tumors resulting from treatment with the test compound (memo Dykstra to Taylor, 2-15-83). Although an apparent increase in the incidence of thyroid carcinoma in treated female rats was noted, no increase in the combined incidence of adenoma and carcinoma was observed, nor was an effect on latency demonstrated. It was concluded by Toxicology Branch that no effect on thyroid was apparent.

E.(4) Correlation of Gross Observations and Histological Findings- In general, the correlation of gross and microscopic findings was good. The majority of gross observations for which no corresponding diagnosis was rendered were for items such as "blood around snout" or "blood around eye" for which a microscopically recognizable lesion would not be likely. Other common gross observations without corresponding lesions included altered color of the kidneys liver, lungs or adrenals, or altered size of various organs. Again, a discrete, microscopically recognizable lesion associated with these observations would not be likely.

Therefore, since few gross observations lacked a corresponding diagnosis, and because no effect of treatment on the incidence of histopathological lesions was apparent, the lack of summary tables for gross observations does impair the analysis of this study.

Discussion

The primary purpose of this DER was to assess the 2-year rat feeding study for evidence that the highest dose tested (HDT) was a Maximally Tolerated Dose (MTD). No effect on clinical signs, body weights, or mortality was noted. No effects on clinical pathology or organ pathology was apparent. Therefore, it is concluded that the HDT was not sufficiently high to be an MTD.

Data Evaluation Record

Chemical: Glyphosate Technical, CP 67573.

Study Identification: "Neurotoxicity Study with Roundup in Chickens"

Accession No.:	229184
MRID No.:	00054494
EPA Reg. No.:	524-EX-29
Study No.:	8580-09117, BTL-76-82 (IBT)
Report date:	12/17/76
Submitted:	Unknown
Sponsor:	Monsanto Agricultural Products Co. St. Louis, MO. 63166
Test facility:	Industrial Bio-Test Laboratories, Inc. Decatur, Illinois 62526
Study authors:	Fletcher, D. and Arceo, R.J.

Reviewed by: D. Stephen Saunders Jr., Ph.D.
Toxicologist, Section V
TOX/HED (TS-769)

Conclusions

No evidence of neurotoxicity. Invalid study due to missing raw data (per validation report), use of animals with disease (see "Discussion").

Classification: Core-Invalid Deficiencies as noted.

Background

This DER is a scientific evaluation of an IBT study that was declared valid after an audit of the raw data supporting this study. The validation report (dated 8/3/78) noted an absence of raw data for dose preparation, body weight measurements, and histopathology in controls, which were common to two other studies. In spite of these deficiencies, the validation report considered the study to be valid.

Materials and Methods

A. Materials: (1) Test Material- Technical glyphosate (CP 67573), lot QH-68, 94% a.i.

(2) Doses Tested- 1.25 g/kg two times/day days 1-3 and days 21-23, total cumulative dose of 15.0 g/kg over the course of the study. Positive control birds received 500 mg/kg of triorthocresylphosphate (TOCP), however the protocol did not state on what days positive control was administered, or whether it was done concurrently with this study.

(3) Test Animal- Strain and source of hens not stated, 10/group.

(con't)

B. Methods: A photocopy of the submitted methods is appended. Since the study was conducted at IBT, an audit of the raw data supporting this study was performed, and the following deficiencies were noted in the validation report (dated 8/3/78):

(1) No records of dose preparation or administration were available.

(2) No records of body weight measurements were available.

(3) No records of pathological observations were available for untreated or positive control birds. The control groups were evidently shared with other studies, however it was not stated whether these birds were treated concurrently with birds administered the test article.

Results

A. Body Weights- Data for this parameter were submitted in the study report as individual animal values on days 0, 21, and 42 for untreated control and test birds. The validation report narrative noted an absence of raw data for body weight determinations.

The study report indicated that over days 0-21, 9/10 treated birds lost body weight, and average weight gain in these birds was -104.0 ± 113 grams. Weight gain in controls was 77.5 ± 89.5 grams, and 9/10 birds gained weight over this period. Over days 21-42, average weight gain in treated birds was 95.0 ± 85.2 grams, and 8/10 birds gained. Weight gain in controls over days 21-42 averaged 11.5 ± 37.3 grams, and 6/10 birds gained weight over this period.

B. Clinical Signs and Mortality- The validation report noted that "No new toxicity grading data [for daily observations] were presented although data for the untreated control were available". No data for clinical signs were submitted with the study report, however the report narrative stated that "test and control birds appeared normal throughout the 42-day test period....No mortalities occurred in the test or control groups".

C. Necropsy- The validation report did not discuss the raw data for gross observations at necropsy. Data for gross observations were not submitted with the study report, however the report narrative stated that "gross pathologic examination of all animals at the time of sacrifice revealed no abnormal tissue alterations".

Histological examinations were confined to "representative specimens of brain, spinal cord, and sciatic nerve of the untreated control, positive control, and test groups". Data were submitted in the study report as individual animal findings for 10 untreated control, 4 positive control, and 10 treated birds. The raw data validation report noted an absence of raw data for histopathological examinations in untreated and positive control animals, and stated that they were common to two other studies (J9116 and J9120). It was not clear from the submitted methods or the validation report whether the control animals were treated concurrently with the test animals.

The submitted data demonstrated axonal degeneration and demyelination of the spinal cord and sciatic nerve in positive control (TOCP) birds only. Perivascular lymphoid infiltration was noted in the brain, spinal cord, and sciatic

nerve, and interstitial lymphoid infiltration of the sciatic nerve were noted in all test animals without relationship to treatment with the test article. The report narrative stated that the lymphoid infiltrates were due to "lymphomatosis (Marek's Disease), a naturally occurring viral disease of chickens". The 1982 Pesticide Assessment Guidelines state that "Healthy animals shall be used...". The presence (in control and treated birds) of a disease affecting the nervous system, the target organ in the present study, complicates the interpretation of the study and is considered to be a major deficiency.

Discussion

The test article did not appear to induce a delayed neurotoxicity syndrome in hens. The raw data validation noted an absence of supporting documentation for dose preparation and administration, body weight measurements, and histopathological examinations.

Classification: Core-Invalid Deficiencies as noted above and in "Methods".

D. Fears 5/13/81 Review, 1

HEN. Neurotox

III. Procedure

The test utilized 1 control group and 1 test group, each group consisting of 10 hens. All birds were over 9 months old and were under observation for a pretest period to determine their suitability as test animals based on their general physical condition. The birds were housed in standard pens and maintained on Purina Flock Chow* with water offered ad libitum.

Following a 16-hour fast, the test material** was administered to each animal at a rate of 1.25 g/kg*** of body weight. Previously calculated doses of neat material were volumetrically measured and administered via gavage to each individual animal. Dosing was carried out twice daily (b.i.d.) for 3 consecutive days. The dosing procedure was repeated on the 21st day. The total dosage of test material administered to each chicken was 15.0 g/kg. Also, 1 group of birds received a dosage of Triortho Cresyl Phosphate (TOCP) known to produce delayed neurotoxicity, i.e., 500 mg/kg.

The birds were observed daily for mortality and possible neurotoxic reaction for the 42-day test period. Body weights were recorded at 0, 21 and 42 days. At the end of the test period, all surviving birds were sacrificed and subjected to a gross pathological examination. The brain, sciatic nerve and spinal cord were removed in situ and fixed in 10% buffered formalin.

* Ralston Purina Company, St. Louis, Missouri.
** The test material was diluted with water prior to administration.
*** Dosage was established by initial range finding. Range finding was started at 5.0 g/kg twice a day for 3 consecutive days and was reduced by 50 percent for each successive dose until a non-lethal level was determined.

Data Evaluation Record

Chemical: ^{14}C-Glyphosate Technical, CP 67573.

Study Identification: "The Gross Metabolism of N-phosphonomethylglycine-^{14}C in the Laboratory Rat Following a Single Dose".

Accession No.:	Unknown
MRID No.:	Unknown
EPA Reg. No.:	524-308
Report No.:	297 (Monsanto)
Report date:	6/15/73
Submitted:	Unknown
Sponsor:	Monsanto Commercial Products Co. St. Louis, MO. 63166
Test facility:	Monsanto Agricultural Division, Research Department
Study authors:	Colvin, L.B. and Miller, J.A.

Reviewed by: D. Stephen Saunders Jr., Ph.D.
Toxicologist, Section V
TOX/HED (TS-769)

Conclusions

Greater than 95% excretion of label by 120 hours after treatment. 15% eliminated in urine for males, 40% for females. Minimal retention in tissues.

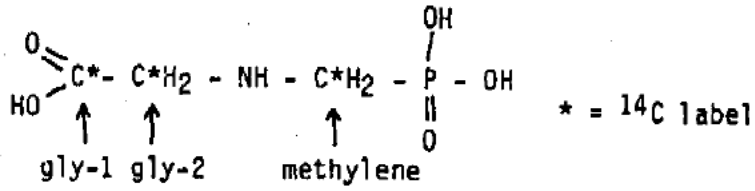
Classification: Core-Supplementary Deficiencies as noted in "Methods" and "Discussion".

Background

This study was originally submitted to Residue Chemistry Branch under PP#4G1444. Although these data were reviewed as part of the RCB chapter for the Glyphosate Registration Standard, they are reviewed here since a rat metabolism study is necessary to satisfy toxicology testing requirements.

Materials and Methods

A. Materials: (1) Test compound- ^{14}C-Glyphosate (N-phosphonomethylglycine), labelled in the methylene (CH_2) position (8.03 mCi/mmole), or in the 1 (gly-1, carboxyl) or 2 (gly-2, alpha) position of the glycine moiety (10.02 and 9.40 mCi/mmole, respectively). Structure and location of labels (acid form):



(2) Doses tested- Approximately 6.7 mg/kg by gastric intubation or 2.33-3.63 mg/kg by intraperitoneal injection.

(con't)

(3) Test animal- Male and female Wistar (SPF) rats, obtained from National Laboratory Animal Co., Creve Coeur, MO. Refer to results sections for the number of animals used for each experiment.

B. Methods: A photocopy of the submitted methods is appended. The following point(s) are noted:

(1) Only 1-3 animals per test group were studied, in contrast to the 1982 Pesticide Assessment Guidelines which recommend 5/sex in each treatment group.

(2) In contrast to current guidelines, excreta were apparently not analyzed for the presence of metabolites.

(3) In contrast to current guidelines, the effect of a minimally toxic dose on excretion and metabolism (Group D of the guidelines) was not assessed.

Results

A. Excretion After an Oral Dose- The clearance of the gly-1 and -2 labels was studied in three males each, and the CH_2 label was administered to 2 males. In females, the gly-1 and CH_2 labels were given to 1 rat each and the gly-2 label was given to two females.

Elimination of an adminstered oral dose (a.o.d.) was between 98 and 100% in males and 93-95% in females by 120 hours after treatment. The molecular location of the ^{14}C label had little effect on the total %a.o.d. excreted or the distribution of label between urine and feces. (The single female given the CH_2 label died after 60 hours; total excretion for this animals was 88% a.o.d. at 60 hours). Approximately 15% a.o.d. was excreted in the urine of males, whereas in females urinary excretion accounted for about 35-45% a.o.d. These data suggest that females absorbed about three times as much glyphosate as did males. In either sex, the amount excreted as $^{14}CO_2$ was less than 0.1% a.o.d.

Urinary clearance of the CH_2 labelled glyphosate was directly compared in two males and two females to verify the apparent greater urinary excretion in females. Urinary and fecal clearances in males were 16.2% and 78.7%, respectively, whereas for females these values were 25.1% and 63.8%. Therefore, the apparent greater urinary excretion (and therefore absorption) of glyphosate in females was confirmed.

Data on the identity of excreted metabolites were not included in the study report.

The retention of label (as % a.o.d.) was highest in the muscle and gut of either sex (Table 1), and retention of the glycine labels by these tissues was slightly higher than retention of the methylene label. The highest concentration in a tissue occurred in the liver and kidney (0.1 to 0.2 ppm in females, 0.01 to 0.08 in males) after administration of the gly-1 or 2 labels.

Table 1. Retention of ^{14}C Label in Tissues[a]

	MALES			FEMALES		
	CH^2	Gly-1	Gly-2	CH_2	Gly-1	Gly-2
Liver	0.02[b] (0.01)[c]	0.04 (0.03)	0.08 (0.08)	0.08[d] (0.04)	0.11 (0.10)	0.14 (0.17)
Kidney	0.01 (0.02)	0.01 (0.03)	0.01 (0.08)	0.02[d] (0.05)	0.02 (0.10)	0.04 (0.20)
Muscle	0.05 (<0.01)	0.16 (0.02)	0.29 (0.04)	0.17[d] (0.01)	0.38 (0.05)	0.44 (0.06)
Fat	0.01 (<0.01)	0.04 (0.02)	0.07 (0.04)	0.08[d] (0.03)	0.07 (0.04)	0.12 (0.08)
Gut	0.05 (0.02)	0.07 (0.03)	0.11 (0.04)	0.67[d] (0.14)	0.22 (0.04)	0.25 (0.06)
Blood	<0.01 (<0.01)	0.02 (0.01)	0.03 (0.03)	-[d]	0.05 (0.04)	0.06 (0.06)

[a]data excerpted from submitted study.
[b]% administered dose retained in tissue.
[c]ug ^{14}C equivalents/g fresh tissue.
[d]animal died at 60 hours, residues refer to amount of label remaining at that time, blood not taken.

B. Excretion After an Intraperitoneal Dose- Three males were given an intraperitoneal (ip) dose of one of the labelled test compounds. As was noted after oral dosing, elimination of administered label was virtually 100% after 120 hours. Between 82 and 90% of the administered intraperitoneal dose (a.i.d.) was excreted in the urine, depending on the location of the ^{14}C label. Placement of the ^{14}C label in the methylene portion of the molecule seemed to result in a somewhat greater % excretion in the feces (13.5%), as compared to excretion of test article labelled in the 1 or 2 position of the glycine moiety (7.6% and 5.9%, respectively). Excretion of $^{14}CO_2$ was less than 1% for any of the test compounds, although more of the glycine label (0.7% - 0.8%) was excreted by this route compared to the methylene label (0.05%). These findings suggest that a small amount of metabolism may have occurred in the rat, likely due to cleavage of the the C - N bond, yielding a two carbon fragment and aminomethylphosphonic acid (AMPA). The two carbon fragment would then enter body pools, resulting in generation of CO_2, and the AMPA moiety apparently is preferentially excreted in the bile. However, data on the identity of metabolites excreted in urine or feces were not included in the study report.

Retention of label in tissue after the i.p. injection followed a pattern similar to that observed after oral administration. Muscle and gut apparently retained the highest % a.i.d., reflecting the mass and vascularity of these tissues, and overall retention of label by tissues was higher for the glycine labels compared to the methylene label. (Fat was discounted since the protocol called for sampling fat from the perirenal and epidydimal regions, and artifactually high concentrations of label would be expected due to the intraperitoneal

injection). The highest concentration of label was noted in the liver and kidney of rats treated with the gly-2 label, both of retained about 0.1 ppm of label.

C. Excretion and Distribution of Plant Metabolites- Rats were administered orally the aqueous extracts of roots or tops of soybean plants that had been grown in the presence of one of the labelled test compounds. The chemical identities of the labelled substances were not determined. Of the material from roots, the CH_2 and gly-1 labels were excreted completely by 120 hours, with urinary excretion of 8.8% and 37.6% respectively, and less than 1% of residual label in the carcass. Excretion of the gly-2 labelled root extract was only 86% at 120 hours, with urinary excretion of 31.2%. A significantly higher amount of label was excreted as $^{14}CO_2$ (3.7%), and retained in the carcass of these animals, 5.2% of the administered dose. These findings suggest that catabolism of glyphosate occurs in plants resulting in entry of the alpha carbon (gly-2) into the carbon pool of the plant, resulting in labelling of plant natural products (e.g. amino acids, proteins, and sugars) with ^{14}C. These ^{14}C-labelled natural products were then likely incorporated into the tissues of the rats and/or catabolized to release $^{14}CO_2$.

Excretion of the CH_2-labelled extract from the tops of the plants was also 98% by 120 hours, with urinary excretion of 19.4% and 1.9% residual label in the carcass, and excretion of 3.5% administered dose as $^{14}CO_2$. Since little $^{14}CO_2$ was produced from the CH_2 labelled parent compound, the finding of $^{14}CO_2$ after administration of labelled plant metabolites strongly suggests incorporation of the methylene label by the tops of plants into natural products. Since the tops of plants were not treated with the glycine-labelled test compounds, the degree of incorporation of the glycine moiety by tops cannot be estimated.

Discussion

Glyphosate was shown to be absorbed from the gastrointestinal tract to a greater extent in females than males. Urinary excretion, an index of absorption, was about 15% in males and about 40% of an administered oral dose in females. For both sexes, elimination was virtually 100% by 120 hours. Retention of label in tissues was minimal, as the highest concentration of label occurred in kidneys and liver, but was less than 0.2 ug label/g tissue in all cases. Overall tissue retention in all cases was greater for the glycine labelled materials compared to the methylene label.

The fact that the location of the label produced different results for retention and excretion after oral and i.p. doses strongly suggests that some metabolism of glyphosate occurs in the rat. However, the submitted study does not report any results of analyses for metabolites.

Animals fed extracts of soybean plants grown in the presence of the ^{14}C-labelled test compounds also excreted virtually all of the amount administered, with the exception of animals given extracts of roots grown in the presence of the gly-2 label, and extracts of tops grown with the CH_2 label. The greater retention of these labels would be consistent with catabolism of glyphosate by the plant, resulting in the labelling of natural plant products (e.g. amino acids, proteins, and sugars) which were then incorporated into rat tissues.

Classification: Core-Supplementary (When considered with the data from the repeated dose study, Report #309). Deficiencies as noted in "Methods" and "Discussion".

administration and ^{14}C-activity has been demonstrated in the bile, it is clearly evident that enterohepatic circulation can contribute to the fecal ^{14}C-activity. The label distribution of CP 67573-^{14}C in the feces is Gly-1 > Gly-2 > methylene.

The complete oxidation of CP 67573-^{14}C to $^{14}CO_2$ does not constitute a major route of metabolism in either sex. Even after intraperitoneal injection less than 1% of the dose was expired as $^{14}CO_2$.

The tissue retention of CP 67573-^{14}C is slightly larger in female rats than in males. The residue from both glycine labeled materials are found largely in the muscle, whereas, the methylene label is secreted into the gut.

When the extracts of soybeans which have been grown in hydroponic solutions of CP 67573-^{14}C are orally administered to male rats, clearance is 96-99% complete in 48 hours except for extracts of roots from Gly-2 treatments which were only 75% cleared in 48 hours. The relatively high tissue retention and $^{14}CO_2$ expiration by rats administered the extracts of Gly-2 roots and methylene tops are undoubtedly due to metabolism of natural plant products, since the ^{14}C-activity contained in those extracts was due to 30% and 10% natural products, respectively (Appendix D and ref. 27).

V. EXPERIMENTAL METHODS

All gross distribution experiments were conducted with Wistar origin, SPF rats (National Laboratory Animal Company, Creve Coeur, Missouri) with initial weights of approximately 150 g, and all preparations of CP 67573-^{14}C ranged from 95-98% in both chemical and radio purity (17).

Following a four hour fast the animals were administered aqueous solutions of CP 67573-^{14}C by gastrointubation and placed in individual Roth-metabolism units (18) permitting separate collection of urine, feces and expired gases. Feed and water was allowed ad libitum. The animals were then maintained for 120 hours as described by Colvin and Miller (19).

The gross distribution of CP 67573-^{14}C was determined by conducting two replicate experiments in which the ^{14}C-activity from CP 67573-^{14}C was assayed in the excreta, expired CO_2 and tissues of eight male rats following a single oral dose of approximately 1 mg (6.7 mg/kg body weight).

Two rats were administered CP 67573-^{14}C labeled in the methylene position (CP 67573-$^{14}CH_2$, 8.03 mCi/mM); and three each were administered CP 67573-^{14}C labeled in either the carboxyl (CP 67573-Gly-1-^{14}C, 10.02 mCi/mM) or the alpha carbon (CP 67573-Gly-2-^{14}C, 9.40 mCi/mM) of the glycine moiety,

respectively. The excreta and expired CO_2 from each animal was collected and assayed for ^{14}C-activity for 120 hours post-administration (p.a.) as previously described (19). After the 120 hour sampling period, a heparinized blood sample was taken by cardiac puncture under ether anesthesia and the animals sacrificed by ether anesthesia. Tissue samples were quickly removed, weighed wet and frozen in an acetone-CO_2 bath for lyophilization. The dry tissues were weighed, pulverized and 200 mg aliquots were combusted by the method of Peterson (20). The resulting trapped $^{14}CO_2$ was then assayed by liquid scintillation analysis.

The percent of the dose recovered from the muscle, adipose tissue and blood were calculated from:

$$\% \text{ dose recovered} = \frac{\% \text{ tissue in body} \times \text{body wt.}}{\text{wt. of wet tissue sample}} \times \frac{\text{dpm in sample} \times 100}{\text{dpm in dose}}$$

The values for the total muscle mass were calculated on the assumption that skeletal muscle comprises approximately 38% of the total bulk of the body in the rodent (21). The percent recovered in adipose tissue was derived from data compiled from laboratory albino mice, under the assumption that rats will not significantly differ from mice in body composition. Total body fat was assumed to be 9.2% of the "corrected live weight," (CLW), i.e., the gross body weight less the weight of gut content and fur. Actual values however were calculated substituting gross body weight for CLW (22).

Blood was calculated as the whole blood volume of 54 ml/kg body weight (23).

The "Gut" samples taken represent the entire gastrointestinal tract, including contents, from the lower esophagus to the rectum just proximal to the anus and therefore included the cecum and its contents. All other tissues represent the whole organ.

The "ppm CP 67573-^{14}C equivalents" were calculated assuming that the total residue recovered in any given tissue is represented by a compound of the same molecular weight as the parent compound. Therefore the calculations were as follows:

$$\text{ppm CP 67573-}^{14}\text{C equiv.} = \frac{\text{dpm/g tissue}}{(\text{dpm}/\mu\text{Ci} \times \mu\text{Ci}/\mu\text{M CP 67573})/(\mu\text{g}/\mu\text{M CP 6757}}$$

The effect of parenteral dosing was determined by the intraperitoneal injection (i.p.) of each of three rats with Gly-1-^{14}C, Gly-2 ^{14}C or $^{14}CH_3$-labeled CP 67573, respectively. The excreta and CO_2 from each animal was collected and assayed for ^{14}C-activity for 120 hours post-administration. At 120 hours p.a. a blood sample was taken, the animals sacrificed and tissue samples quickly removed for ^{14}C-residue analyses.

Biliary secretion of CP 67573 was determined by fistulation of the common bile duct (ductus choledochus) of 300 g rats with a cannula of polyethylene tubing, I.D. 0.011 in. x O.D. 0.024 in. (Intramedic, PE-10, Clay Adams, Parsippany, N.J.) under pentobarbital anesthesia. The bile was collected at 30 minute intervals using an LKB fraction collector. CP 67573-$^{14}CH_2$ was then administered by intragastric infusion, intraperitoneal injection or intravenous injection.

To determine the gross distribution of the plant-derived metabolites of CP 67573, aqueous plant extracts were administered orally. The extracts were derived from soybean plants which had been cultured for four weeks in separate hydroponic media containing each of the ^{14}C-labeled CP 67573 compounds. In each of two replicate experiments one rat was administered the extract of soybean roots from plants treated with CP 67573-Gly-1-^{14}C, -Gly-2-^{14}C or $^{14}CH_2$, respectively. In addition one animal in each replicate was administered the extract of the aerial portion of the soybean plants treated with CP 67573-$^{14}CH_2$.

The animals were individually maintained for 120 hours post-administration during which time the excreta and CO_2 were collected and assayed. At the termination of the experimental period, blood and tissue samples were taken for ^{14}C-analyses.

In a third experiment the rats were also dosed per os with plant extracts but were sacrificed at 24 hours post-administration, and the tissue samples analyzed for ^{14}C-activity.

VI. RESULTS

A. Gross distribution of ^{14}C-activity in rats orally administered CP 67573-^{14}C. The gross distribution of ^{14}C-activity from male rats (Table I) shows that rats administered CP 67573 labeled in the glycine moiety expired only 0.5% of the oral dose (o.d.) as $^{14}CO_2$ and lesser amounts (0.02%) when the label was in the methylene position.

The total urinary clearance of CP 67573-^{14}C was 14% o.d. when labeled in the glycine moiety and 16% o.d. when labeled in the methylene position, with the balance (81-85%) appearing in the feces. Carcass retention at 120 hours p.a. was 0.65%, 0.37% and 0.14% o.d. for the Gly-2-^{14}C, Gly-1-^{14}C, and $^{14}CH_2$ label, respectively. Total accountability of ^{14}C-activity ranged from 98-101% for all labels.

The distribution of ^{14}C-activity in the excreta of female rats following a single oral dose (Table II) exhibited a remarkably different partition between urine and feces than that seen in male rats. Female rats excreted 35-43% o.d. in the urine compared to 14-16% by the males.

Chemical: ^{14}C-Glyphosate Technical, CP 67573.

Study Identification: "The Dynamics of Accumulation and Depletion of Orally-Ingested N-phosphonomethylglycine-^{14}C".

Accession No.:	Unknown
MRID No.:	Unknown
EPA Reg. No.:	524-308
Report No.:	309 (Monsanto)
Report date:	10/73
Submitted:	Unknown
Sponsor:	Monsanto Commercial Products Co. St. Louis, MO. 63166
Test facility:	Monsanto Agricultural Division, Research Department
Study authors:	Colvin, L.B. and Miller, J.A.

Reviewed by: D. Stephen Saunders Jr., Ph.D.
Toxicologist, Section V
TOX/HED (TS-769)

Conclusions

Steady-state for accumulation and excretion of label by day 8. Less than 0.1 ppm of label in tissues 10 days after last dose.

Classification: Core-Supplementary Deficiencies as noted in "Methods" and "Discussion".

Background

This study was originally submitted to Residue Chemistry Branch under PP#4G1444. Although these data were reviewed as part of the RCB chapter for the Glyphosate Registration Standard, they are reviewed here since a rat metabolism study is necessary to satisfy toxicology testing requirements.

Materials and Methods

A. Materials: (1) Test compound- ^{14}C-Glyphosate (N-phosphonomethylglycine), the molecular location of the label, specific activity, and purity were not provided.

(2) Doses tested- 0, 1, 10, and 100 ppm of ^{14}C-glyphosate in the diet for a maximum of 14 consecutive days.

(3) Test animal- Male and female Wistar (SPF) rats, obtained from National Laboratory Animal Co., Creve Coeur, MO. 12/sex for control, 16/sex/dose for treated groups.

(con't)

B. Methods: A photocopy of the submitted methods is appended. The following point(s) are noted:

(1) This study was conducted in 1973, before the current Pesticide Assessment Guidelines were written. The submitted study was conducted in a manner analogous to the methods recommended under present guidelines for the 14 day repeated dose study.

(2) Data for excretion of label were not submitted.

(3) Data for analysis of blood and/or excreta for metabolites were not reported.

(4) Data for body weight gain were not submitted.

Results

A. Body Weights- No effect of treatment on body weight gain was reported. Actual data for body weight gain were not submitted.

B. Organ Weights- Relative organ/body weight ratios were calculated for liver, kidney, spleen, heart, brain, and gonads. No effect of treatment on the relative weights of these tissues was apparent.

C. Excretion- Daily urinary excretion of ^{14}C label was reported to average 8-10% of the amount ingested, without relation to dose or sex. Actual data were not submitted, however several figures listed in the Table of Contents were omitted from the study submitted for review.

A steady-state for urinary and fecal excretion was reached by about 8 days of treatment, indicating that at this time the amount excreted was approximately equal to the amount ingested. By day 14, the total amount excreted was >90% of the amount ingested for all dose groups. After withdrawal of treatment, the amount of label excreted in urine or feces declined rapidly until about day 4, when a slight plateau representing redistribution of label from tissues stores was observed.

D. Tissue Residues- Accumulation of label in tissues was minimal, and a steady-state for accumulation of residue was reached in most cases by around day 8. The fact that an equilibrium for accumulation of residues is attained suggests that glyphosate poses little hazard for bioaccumulation, as is further supported by the steady decline in tissue residues over the 10-day period following withdrawal of the test diets. At the end of the withdrawal period, residues remaining in tissues were generally 0.1 ppm (ug ^{14}C equivalent/g tissue) or less in animals given diets of 100 ppm, with no apparent sex differences. When expressed on the basis of dry tissue weight, residues in the tissues of high dose (100 ppm) rats ranged from a low of 0.2 ppm in brain to a high of 0.42 in the testes.

Discussion

The submitted data demonstrate that the test article poses little hazard for bioaccumulation. The amount of residue accumulated in tissues reached a steady-state equilibrium, as did excretion of label. After withdrawal of the test diets, the amount of label excreted also declined rapidly. Actual residues of label remaining in tissues 10 days after the last dose were generally 0.1 ppm or less on a wet organ weight basis.

Classification: Core-Supplementary When considered with the data from the single dose study (Report #297). Registrant should provide details as to the chemical and radio purity of the test article, and the molecular location and specific activity of the ^{14}C label, and the missing data as identified in the Methods section of this review.

V. EXPERIMENTAL METHODS

A. Experimental Animals and Maintenance

One hundred twenty (60 ♂ and 60 ♀) Wistar-strain, SPF, albino rats (National Laboratory Animals, Creve Coeur, Missouri) were weighed and randomly distributed into individual stainless-steel, rodent metabolism study cages (Figure 1) (Acme Research Products Co., Cincinnati, Ohio, Model 640-000), the front and floor of which were constructed of continuous steel rod grid (2 1/2 squares/in.) and enclosed an animal space of 4 1/2 x 8 x 4 1/4 in. Each unit was equipped with a feeder that adjusted to the size of the experimental animal so that the rear quarters were always in the collection area, eliminating food scattering and contamination of the excreta with the experimental diet. Cylindrical food cups and crumb trays facilitated the calculation of food consumption. The collection funnel (Fig. 2) was designed for fast urine runoff and efficient urine-feces separation.

The animals were acclimated to these cages for seven days during which time a rigid feeding schedule was established. A weighed portion of feed was provided at 0900 in the tared feed cup and removed at 1600. The amount of feed consumed was determined by difference after collecting and combining the feed spilled in the crumb tray with that remaining in the feed cup. Water was allowed ad libitum.

After the seven-day pre-experimental period all animals were weighed and assigned to treatment groups. During the twenty-four day experimental period, feed consumption was recorded on a daily basis in order to calculate daily intake of CP 67573-^{14}C. Urine volumes and feces weights were also recorded daily. The urine, which was collected in a graduate cylinder under the collection funnel, was removed at 0900 daily, the volume recorded, transferred to a 4 or 10 drum, screw-cap vial and frozen until time for sampling. The feces was transferred to a tared four oz. screw-cap bottle and frozen.

Individual weights were determined for all surviving animals at seven day intervals and terminal weights were taken at the time of sacrifice of each animal. Daily observations were made for gross behavioral changes and at necropsy observations for gross pathological changes were made.

B. Experimental Design

In order to obtain data on both the accumulation and depletion of tissue residues, three treatment levels in addition to a control diet, were fed for 14 days after which all remaining animals were fed the control ration.

The three levels of CP 67573 incorporation fed were logarithmic increments of the contemporary projection of anticipated plant residues of CP 67573. The treatment levels which were designed to generate a dose-response curve from a low to an exaggerated level, are shown below:

Treatment	Number of Animals ♂	♀
a. Control (untreated)	12	12
b. 1 ppm CP 67573	16	16
c. 10 ppm CP 67573	16	16
d. 100 ppm CP 67573	16	16

Each treatment was divided into replicate groups of four animals of each sex and distributed into five cage batteries in a randomized block design (Fig. 3). At each sampling period, two animals of each sex from each treatment group were sacrificed and tissues taken for analysis. Animal selection was by a table of random numbers. The sequence of animal scarifice and the sampling periods are shown in Table 1. Due to the limitation of 120 metabolism units, only 12 control animals were used. Therefore no control animals were sacrificed at "2 days medication" and "1 day withdrawal".

C. Analysis of Excreta and Tissues

Duplicate 0.2 ml aliquots of the daily individual urine samples were diluted with 2 ml 0.5M NH_4HCO_3 and the ^{14}C-activity determined in a Mark I Liquid Scintillation Spectrometer (Nuclear-Chicago, DesPlaines, Illinois) in 15 ml Insta-Gel phosphor solution (Packard Inst. Co., Downers Grove, Illinois). Quench corrections were made by automatic external standardization and calculations were facilitated by direct interfacing of the instrument to a CDC 1700 computer.

Daily individual feces samples were homogenized in 20 ml of aqueous 30% isopropyl alcohol using a Brinkman Polytron (Brinkman Inst., Westbury, N.Y.). The homogenate was frozen, lyophilized, weighed and duplicate 100 mg aliquots were submitted for combustion by the method of Peterson, et.al (22-23). The resulting $^{14}CO_2$ was trapped and analyzed by liquid scintillation counting.

At each sampling period each animal to be sacrificed was anesthetized with ether, a heparinized blood sample removed by cardiac puncture, and the animal sacrificed by ether anethesia. Immediately upon death the following tissues were quickly removed, weighed, and quick-frozen:

1. Liver
2. Kidney
3. Heart
4. Spleen
5. Gonads
6. Brain
7. Muscle (striated)
8. Adipose (composite-abdominal and supra-renal)
9. Gut (from lower esophagus to anal sphincter including cecum. Contents of the entire gut were included.)

The frozen tissues were then lyophilized, weighed, ground and duplicate 200 mg. aliquots submitted for combustion analysis. ^{14}C-Activity was determined by liquid scintillation counting of the resultant trapped $^{14}CO_2$.

D. Statistical Evaluation

The limitation imposed by the number of available metabolism units was taken into account when statistical analyses were made. Although relative organ weights were obtained on organs from all animals at each sampling period, statistical comparisons were made only among those samples which would include control values, i.e., 2-days medication and 1-day withdrawal were omitted. Therefore, the relative organ weight was a 2 x 4 x 6 factorial (sex x level x time). However, since a physiological change with time was expected, only a two-way analysis of variance (AOV) was conducted in order to increase the sensitivity for detecting treatment differences.

The residue and excretion portions of the experiment were 2 x 4 x 8 and 2 x 4 x 24 factorials, respectively. However, since the differences between the controls, 1 ppm, 10 ppm or 100 ppm in either residue or excretion of CP 67573 were obvious from the data, it was only necessary to compare sexes.

In the case of relative tissue residues, i.e., tissue to blood residue ratios, the control animals which had no residues were omitted from the analysis for the ease of computations. Relative tissue residues were then analyzed for sex differences independent of the two-way AOV for treatment differences.

The values reported in Tables 2-7, 9-18, 20-28 and 30-39 are the mean of two animals of each sex at each sampling period. The accumulation depletion curves (Fig. 14-21) were plotted from the mean of tissues from two males and two females ± one standard deviation. The percent cumulative intake curves (Fig. 11-13) were constructed from the mean of all rats (♂+♀) surviving at that time, but the µg excreted curves (Fig. 7-10) are plotted as the mean of surviving members of each sex ± one standard deviation.

The calculation of standard deviations was facilitated by use of the Olivetti-Underwood Programma 101 using program 1.50 (22).

In cases in which sex comparisons were indicated, comparisons were made by calculation of the t-statistic (23) and the significance determined from a table of critical values for the "Students" t-statistic for non-directional (two-tailed) tests (24).

The AOV used was a two-way factorial analysis adapted from Bruning and Kintz (24) and programmed into the Com-Share system by Mr. John T. Moran. Statistical significance was determined from a table of F-distribution of mean-square ratios (24).

VI. RESULTS

A. Body and Organ Weight Changes

The male rats when received weighed approximately 125 g and the females of the same age were approximately 5 g lighter. The animals were weighed again at the initiation of the experimental period and weekly thereafter. The growth curves and weight changes are shown in Figures 4 and 5, respectively. Similar growth patterns were exhibited by all groups except the control female rats, which showed a premature growth plateau (Fig. 4). A few animals showed a slight negative response to the individual housing and rigid feeding schedule during the adaptation period. However, all animals except the control females quickly overcame the temporary lag in growth and grew normally throughout the experimental period (Fig. 5).

Although extrinsic stress is often evidenced by anorexia and subsequent weight loss, an intrinsic stress may be manifest in organ hypertrophy or atrophy. Therefore the weight of the fresh organs of the animals were compared to those of the control animals sacrificed at the same sampling period. To correct for the individual weight differences among rats the weights of the organs were expressed as a percent of the live body weight of the individuals (relative organ weight, Tables 2-7). The statistical comparisons of relative organ weights (Table 8) of animals receiving CP 67573-^{14}C versus control animals were restricted to 6, 10 and 14 days medication, and 3, 6 and 10 days withdrawal, because no control animals were sacrificed on days 2 medication and 1 withdrawal. However, the relative organ weights of treated animals sacrificed on those days are included for completeness. The two-way analysis of variance demonstrates that there was no significant change in the size of the liver, kidney,

Data Evaluation Record

004165

Chemical: N-nitroso-glyphosate (sodium salt), CP 76100; 19.8% a.i.

Study Identification: "Two-Year Oral Toxicity Study with CP 76100 in Albino Rats".

Accession No.: 247745-52
EPA Reg. No.: 524-308
Study No.: 8560-08924 (IBT)
Report date: 5/14/79
Submitted: 6/24/82
Sponsor: Monsanto Agricultural Products Co. St. Louis, MO. 63166
Test facility: Industrial Bio-Test Laboratories, Inc. Decatur, Illinois 62526
Study authors: Leslie D. Morrow, et al.

Reviewed by: D. Stephen Saunders Jr., Ph.D.
Toxicologist, Section V
TOX/HED (TS-769)

Background

The study was conducted with the sodium salt of N-nitroso-glyphosate, which is a contaminant of the herbicide glyphosate. The Registrant apparently initiated this study because of concerns over the potential toxicity of the nitroso contaminant. Because this study was conducted at IBT, an audit of the raw data was performed. Based on the findings of that audit, the study was classified as Supplementary data due to deficiencies in supporting raw data for dose preparation, physical observations, and organ weight measurements.

Discussion/Conclusions

An appropriate control group was not used in this study. Because the test article was supplied as a sodium salt, the investigators attempted to treat control rats with an amount of sodium equivalent to that given high dose animals. An error in calculation resulted in control animals apparently receiving 30 mg/kg/day of NaCl, as reported on page 10 of the report narrative. This amount was reported by the investigators to be 4 times the amount of sodium that high dose rats received. The amount of salt given controls appears to have had a toxic effect. Survival was lowest in male and female control groups compared to treated animals, as tabulated below:

	MALES Month			FEMALES Month		
Dose	12	18	24	12	18	24
0	46/60[a] (77%)	38/60 (63%)	10/60 (17%)	48/60 (80%)	39/60 (65%)	16/60 (27%)
3	56/60 (93%)	50/60 (83%)	26/60 (43%)	59/60 (98%)	54/60 (90%)	28/60 (47%)
10	57/60 (95%)	48/60 (80%)	18/60 (30%)	57/60 (95%)	52/60 (87%)	33/60 (55%)
30	54/60 (90%)	41/60 (68%)	17/60 (28%)	57/60 (95%)	46/60 (77%)	32/60 (53%)

[a]number alive/number on test, does not include interim sacrifices.

The control group also had the lowest average body weight gain, compared to test groups, as evidenced by the 24-month average weight gain (grams ± std. dev.):

Dose	Male	Female
0	402 ± 92	249 ± 72
3	441 ± 91	302 ± 89
10	465 ±119	326 ± 70*
30	457 ± 76	325 ± 84*

*$p < 0.05$

Therefore, it is not possible to assess the effect of the test article on treated animals. No effect of treatment on the incidence of neoplasms was apparent, however it cannot be determined whether the doses tested were sufficiently high to detect an oncogenic effect. Approximately 10% decreases in erythrocyte count, hemoglobin content and hematocrit were noted in high dose females at 18 and 24 months, however it is not clear whether this apparent effect was the result of changes in the control group or in the treated animals.

The study is therefore compromised due to the lack of an adequate control group, and is considered to be invalid.

Classification: Core-Invalid Inappropriate control group.

Data Evaluation Record

Chemical: N-nitroso-glyphosate (sodium salt), CP 76100.

Study Identification: "Two-Year Chronic Oral Toxicity Study with CP 76100 in Dogs".

Accession No.:	247753
EPA Reg. No.:	524-308
Report No.:	8580-08922 (IBT)
Report date:	5/8/79
Submitted:	6/24/82
Sponsor:	Monsanto Agricultural Products Co. St. Louis, Missouri 63166
Test facility:	Industrial Bio-Test Laboratories, Inc. Decatur, Illinois 62526
Study Authors:	Donald H. Jenkins, et al.

Reviewed by: D. Stephen Saunders Jr., Ph.D.
Toxicologist, Section V
TOX/HED (TS-769)

Conclusions

LEL = 30 mg/kg/day Increased absolute and relative kidney weights.

NOEL = 10 mg/kg/day by gavage

Classification: Core-Supplementary Per validation report.

Background

The study was conducted with the sodium salt of N-nitroso-glyphosate, which is a contaminant of the herbicide glyphosate. The Registrant apparently initiated this study because of concerns over the potential toxicity of the nitroso contaminant. Because this study was conducted at IBT, an audit of the supporting raw data was performed as part of the Agency's validation process for IBT studies (Dynamac contract no. 68-01-6561, accepted by EPA 6/5/85). Based on the findings of that audit, the study was classified as Supplementary data due to deficiencies in the supporting raw data (see "Methods").

Materials and Methods

A. Materials: 1) Test material- N-nitroso-glyphosate, sodium salt; CP 76100; Lot # T-107; the % a.i. was not stated.

2) Doses tested- 0, 3, 10, and 30 mg/kg/day via gelatin capsule.

3) Test animal- Purebred beagle dogs, obtained from IBT breeding colony.

(con't)

B. Methods: The methods used and all supporting raw data were reviewed as part of the EPA validation process for IBT studies. The validation report found that raw data were lacking for gross observations at necropsy, ocular examinations, clinical observations, and for preparation of test doses. The validation report concluded that the study could only be considered as Supplementary data.

Other than the lack of supporting raw data, no deficiencies in the methods used were noted.

Results

A. Clinical Signs and Mortality- The only clinical observations reported were for a single male of the low dose group (3 mg/kg) who had a "minor respiratory infection" at week 16, and was treated successfully with an antibiotic. It was noted in the audit validation, however, that raw data supporting physical examinations were lacking.

No deaths were reported.

B. Body Weights and Food Consumption- No treatment-related effects on body weight gain or food consumption were noted.

C. Clinical Pathology: (1) Hematology- Apparently treatment-related decreases of about 10% in erythrocyte count, hemoglobin concentration, and hematocrit were noted in high dose male and female dogs at 24 months. Other parameters were unaffected.

(2) Serum Chemistries- No effect of treatment on BUN, SGOT, SGPT, or SAP was apparent. An apparent increase in serum glucose of about 10% was noted in high dose females that appeared to be treatment-related.

(3) Urinalysis- No effect of treatment on these parameters was apparent.

D. Ophthalmological Examinations- No effect of treatment on the incidence of eye lesions was apparent. However, it was noted in validation report for this study that raw data for these examinations was lacking.

E. Necropsy Data: (1) Organ Weights- Although occassional alterations in absolute organ weights were noted, these generally could be attributed to fluctuations in body weight. The only organ for which relative weights were altered was kidney. Absolute kidney weight, kidney/body and kidney/brain weight ratios were increased in high dose females by about 25%.

(2) Gross Observations- The report narrative stated that the only apparent treatment-related change was enlarged spleen, observed in high dose males. This lesion was noted in 1/4 control, 0/4 low and mid dose, and 2/4 high dose males. However, the absolute spleen weights of the affected animals were not different from control. Therefore the significance of this apparent finding is unclear.

The validation report of the raw data audit questioned the adequacy of gross examinations because no gross observations were recorded for 3/8 control and 8/24 treated dogs.

(3) Histopathology- No effects of treatment on organ histology were noted. Common findings without apparent relation to dose included "few degenerate glomeruli" and focal or diffuse congestion of the spleen. No effects in the kidney were reported that correlated with the apparent increase in absolute and relative weights that was noted in high dose females.

Discussion

The only apparent treatment-related effect was an increase in absolute and relative kidney weights in high dose females. This finding, however, was not supported by a corresponding effect on gross or microscopic observations, or on BUN, an index of kidney function. An increase in blood glucose in high dose females could indicate altered kidney function, however. Therefore, the apparent increase in kidney weight is of uncertain toxicological significance.

LEL = 30 mg/kg/day Increased absolute and relative kidney weights, increased blood glucose in females.

NOEL = 10 mg/kg/day

Classification: Core-Supplementary Per raw data audit validation report: raw data missing for preparation of test doses, clinical observations, gross observations at necropsy, and ocular examinations.

Pages 52-57 -* Claimed confidential by submitter*

Data Evaluation Record

Chemical: ^{14}C-N-Nitroso-Glyphosate, CP 76000.

Study Identification: "Metabolism of N-Nitroso-phosphonomethylglycine in the Laboratory Rat".

Accession No.:	233913
MRID No.:	Unknown
EPA Reg. No.:	524-308
Report No.:	MSL 0242 (Monsanto) RD #179
Project No.:	7863
Report date:	4/26/78
Submitted:	Unknown
Sponsor:	Monsanto Commercial Products Co. St. Louis, MO. 63166
Test facility:	Monsanto Agricultural Division, Research Department
Study author:	Sutherland, W.L.

Reviewed by: D. Stephen Saunders Jr., Ph.D.
Toxicologist, Section V
TOX/HED (TS-769)

Conclusions

Approximately 90% of administered label was excreted within 24 hours after a single oral dose, with the majority of elimination in the urine. Similar results were obtained after 5 consecutive doses, however only 60% of the administered label was excreted in the urine, apparently due to the higher doses used in the multiple dose study. Minimal retention in tissues after repeated doses.

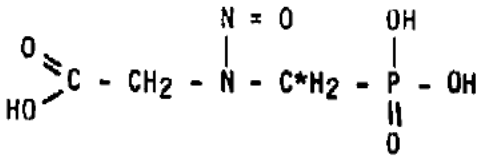
Classification: Core-Supplementary Deficiencies as noted in "Methods" and "Discussion".

Background

The study was conducted with the sodium salt of N-nitroso-glyphosate, which is a contaminant of the herbicide glyphosate. The Registrant apparently initiated this study because of concerns over the potential toxicity of the nitroso contaminant.

Materials and Methods

A. Materials: (1) Test compound- $^{14}C/^{13}C$-N-nitroso-glyphosate (N-nitroso-phosphonomethylglycine), 90:10 ratio of isotopes, s.a. of ^{14}C = 10.1 mCi/mmole.



* = $^{14}C/^{13}C$ label

(2) Doses tested- Approximately 1 mg/rat by gastric intubation for the single dose study, or 30 mg/kg in the multiple dose study.

(con't)

(3) Test animal- 3/sex in the single dose study, 2/sex in the multiple dose study; strain, source not provided in test report.

B. Methods: A photocopy of the submitted methods is appended. The methods were reviewed, and the following points are noted:

(1) The same dose was not used in the single and multiple dose studies so that the effect of repeated dosing on excretion and metabolism cannot be readily compared.

(2) The effect of a minimally toxic dose was not assessed.

(3) Only 2-3 animals were used in each test group, in contrast to current guidelines which recommend 5/sex in each group.

(4) A parenteral administration group was not studied.

(5) Tissue residues were not determined in the single dose study.

(6) The protocol followed in the multiple dose study called for 5 consecutive doses and sacrifice on the following day, in contrast to current guidelines which recommend 14 consecutive doses of unlabelled test material followed 24 hours later by a single oral dose of labelled test material.

Results

A. Single Dose- Elimination of label after a single oral dose was rapid, as 84-93% of the administered label was excreted in the urine of males in the first 24 hours, and females excreted 78-83% in the first day. After the first day, none of the animals excreted more than 1% in the urine over the remainder of the 5 day observation period. Fecal excretion of label in males ranged from 1.3-4.3% of the administered dose, and in females was 9.6-11.8%. As was noted for urinary excretion of label, the majority of fecal excretion occurred in the first 24 hours. Thus, the total amount of label excreted by males or females was from 88-96% of the administered dose.

The label that was excreted was the parent compound, N-nitrosoglyphosate (NNG). Transformation of NNG to glyphosate occurred in some urine samples, however it was demonstrated that this conversion resulted from acid hydrolysis of NNG during the concentration step in the processing of urine samples. If samples were neutralized prior to concentration, no hydrolysis of NNG to glyphosate was detected.

The distribution of label in tissues was not assessed in this portion of the study (i.e. after a single oral dose).

B. Multiple Dose- The total excretion of label during the 5 day treatment period was about 92-95% of the administered dose, with no obvious difference between sexes. About 60% of the administered label was excreted in the urine, and the remainder was in the feces.

As was noted in the single dose study, the label was excreted unchanged as the parent compound. A small amount of aminomethylphosphonic acid (<1%) was

detected in some samples, however this was inferred to be an artifact of processing since this metabolite was only detected in urine or feces extracts that had been filtered, and never in un-filtered samples.

Retention of label in tissues was minimal, as the greatest amount of label in a tissue was 0.02% of the total amount administered in the liver. All other tissues (heart, brain, spleen, reproductive organs, kidney, gut, and lung) contained less than 0.01% of the total dose administered. If expressed as ppm, the highest concentration of label was noted in the gut contents (0.8-9.8 ppm), however the gut wall only contained about 0.1 ppm. Retention of label in kidney, spleen and liver averaged 0.26, 0.36, and 0.52 ppm, respectively, with no apparent sex differences. The lung had the highest concentration of label of any organ, ranging from 0.42 to 1.62 ppm with an average of 0.9 ppm. The higher apparent retention of label by the lung may have resulted from the aspiration of test substance during gavage, as was speculated by the investigators.

Discussion

Elimination of NNG appeared to be rapid, as about 90% of an administered oral dose was excreted unchanged in the first 24 hours, with the majority of label appearing in the urine. No difference in excretion rate or the partition of label between urine and feces was noted between males and females.

Similar results were obtained after 5 consecutive doses, as 92-95% of the total administered dose was excreted over the treatment period, with about 60% appearing in the urine and the remainder in the feces. The apparent decrease in the % of label appearing in the urine compared to the single dose study suggests saturation of an absorption pathway with the higher dose used in the multiple dose study. As was noted after a single dose, no metabolism of NNG was apparent, and the label was excreted unchanged.

Tissue retention after 5 doses was minimal, as the highest concentration detected in an organ was an average of 0.8 ppm in the lung, and the concentration of label in kidney, spleen and liver ranged from 0.26 to 0.52 ppm.

Classification: Core-Supplementary Deficiencies as noted in "Methods".

Pages 61-67 -* Claimed confidential by submitter*

Data Evaluation Record

Chemical: ^{14}C-Aminomethylphosphonic Acid, CP 50435.

Study Identification: "The Metabolism of Aminomethylphosphonic Acid-^{14}C in the Laboratory Rat".

Accession No.:	Unknown
MRID No.:	Unknown
EPA Reg. No.:	524-308
Report No.:	303 (Monsanto)
Report date:	8/73
Submitted:	Unknown
Sponsor:	Monsanto Commercial Products Co. St. Louis, MO. 63166
Test facility:	Monsanto Agricultural Division, Research Department
Study authors:	Colvin, L.B., Moran, S.J. and Miller, J.A.

Reviewed by: D. Stephen Saunders Jr., Ph.D.
Toxicologist, Section V
TOX/HED (TS-769)

Conclusions

93% of label excreted unchanged by 72 hours; 20% in urine, 13% in first 12 hours. Only 0.06% of label remained in tissues at 120 hours.

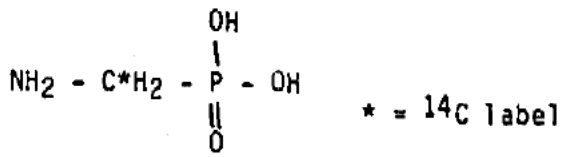
Classification: Core-Supplementary Deficiencies as noted in "Methods" and "Discussion".

Background

This study was originally submitted to Residue Chemistry Branch under PP#4G1444. Although these data on the metabolism by the rat of the plant metabolite aminomethylphosphonic acid (AMPA) were reviewed as part of the RCB chapter for the Glyphosate Registration Standard, they are reviewed here since a rat metabolism study is necessary to satisfy toxicology testing requirements.

Materials and Methods

A. Materials: (1) Test compound- ^{14}C-Aminomethylphosphonic acid (AMPA), 8.9 mCi/mmole. Structure and location of labels:



(2) Doses tested- Approximately 1 mg (6.7 mg/kg) by gastric intubation.

(3) Test animal- Male and female Wistar (SPF) rats, obtained from National Laboratory Animal Co., Creve Coeur, MO. The number of animals used for each experiment was not stated.

(con't)

B. Methods: A photocopy of the submitted methods is appended. The following point(s) are noted:

(1) The number of animals per test group was not stated, and apparently only males were studied. The 1982 Pesticide Assessment Guidelines which recommend 5/sex in each treatment group.

(2) In contrast to current guidelines, the effect of repeated doses was not assessed (Group C of the guidelines).

(3) In contrast to current guidelines, the effect of a minimally toxic dose on excretion and metabolism (Group D of the guidelines) was not assessed.

Results

A. Excretion- The test compound appeared to be rapidly eliminated, as 93% of the administered dose (%a.d.) was excreted by 72 hours. Only an additional 0.6% a.d. was excreted between 72 and 120 hours, when the study was terminated. Total excretion of label in the urine was 20% a.d., with 13% eliminated by this route within the first 12 hours. The remainder of the excretion (73.5%) was via the feces, as only 0.07% was recovered as $^{14}CO_2$. Based on the thin-layer chromatography (TLC), nuclear magnetic resonance (NMR) and mass spectrometry data, the report narrative "stated unequivocally that orally administered CP 50435 [AMPA] is excreted unchanged in the urine of rats". This reviewer generally agrees, however the recovery of $^{14}CO_2$ clearly indicates that a small amount of catabolism of AMPA occurred.

B. Tissue Residues- Little accumulation of the test material was observed. Only 0.06% a.d. remained in the tissues, of which the majority (0.02% a.d.) was in the muscle and gut. These tissues contained the largest total amount of label because of their relatively large mass in the body. The highest concentration of label was also in the gut (0.008 ug/g tissue wet weight, ppm), although kidney and liver both had 0.006 ppm tissue of label. Therefore, the test article appeared to have little potential for bioaccumulation.

Discussion

AMPA was rapidly and completely eliminated from male rats, as about 93% of an administered dose was excreted within 72 hours. Of the 20% a.d. eliminated in the urine, an index of absorption, 13% was excreted in the first 12 hours. Little catabolism of AMPA was noted (0.07% excreted as $^{14}CO_2$), and the investigators stated that the label excreted in urine and feces was the parent compound. A small amount (0.06% a.d.) of label remained in tissues at 120 hours, with majority of label contained in muscle and gut, indicating that bioaccumulation of AMPA was unlikely.

Classification: Core-Supplementary Deficiencies as noted in "Methods".

Of the material absorbed, very small amounts are catabolized as evidenced by the facts that less than 0.1% of the dose is expired as $^{14}CO_2$ and tissue residues are less than 10 ppb.

The high biological stability coupled with the extremely low toxicity of CP 50435-^{14}C indicates that CP 50435 represents no hazard to animal life subjected to acute exposure.

V. Experimental Methods

A. Maintenance and dosing of animals.

Male, SPF, Wister-origin rats (National Laboratory Animal Co., Creve Coeur, Missouri) weighing approximately 150 g were fasted four hours and administered by gastreintubation, an aqueous solution containing approximately 1 mg of CP 50435-^{14}C (8.9 mCi/mM). The animals were immediately placed in individual Roth metabolism units (8) and maintained for 120 hours post-administration as described by Colvin and Miller (10). Feed and water were allowed ad libitum. This corresponds to a dose of 6.7 mg/Kg body weight.

B. ^{14}C-analysis in the excreta and expired gases.

The urine, feces and the 1N NaOH used to trap the expired gases were removed at 12, 24, 48, 72, 96 and 120 hours post-administration. One ml of the NaOH trapping solution was diluted with two ml water, and 15 ml phosphor solution (Insta-Gel® -Packard Instrument Co., Downers Grove, Ill.) added and the sample analyzed for $^{14}CO_2$ by liquid scintillation counting.

The urine was analyzed for ^{14}C-activity by diluting 0.1 ml and 0.2 ml aliquots with four ml 0.1M NH_4HCO_3. Fifteen ml phosphor solution was then added and the mixture shaken vigorously and chilled to form a gel. The samples were then analyzed by liquid scintillation counting.

The feces were removed at each experimental sampling period and immediately frozen until termination of the experiment. The feces samples were then homogenized in 30% aqueous isopropyl alcohol using a Polytron high-speed homogenizer (Brinkmann Instruments, Westbury, N. Y.). The feces homogenate was lyophilized and 100 mg aliquots submitted for combustion by the method of Peterson (11). The trapped ^{14}C-activity was analyzed by liquid scintillation counting.

C. ^{14}C-Residue analysis in tissues.

At the termination of the 120 hour experimental period a heparinized blood sample was taken by cardiac puncture under light ether anesthesia. The animal was then killed by continued ether anesthesia and the following tissues removed: the liver, kidney, spleen, heart, brain, testes, an adipose sample, a voluntary muscle sample and the gut from the esophagus to the rectum including the caecum. The samples were weighed, frozen and lyophilized. The lyophilized samples were weighed, aliquoted and submitted for combustion and subsequent liquid scintillation counting. The calculations of the percent of dose recovery in muscle, fat and blood, and the ppm calculations were made under the assumptions described previously (7).

D. Isolation and identification of CP 50435-^{14}C.

1) Preparation of analytical standards of CP 50435-^{14}C derivatives.

Standard samples of CP 50435-^{14}C of 50-400 µg were lyophilized in 3.5 ml screw-cap vials with self-sealing teflon coated diaphragms (No. 915, Schwartz-Mann, Orangeburg, N.Y.). To each vial was added 0.5 ml trifluoroacetic acid followed by 0.5 ml trifluoroacetic anhydride. The reaction was allowed to proceed at room temperature for one hour with occasional swirling. The reaction mixture was evaporated to dryness in an 80-90° C sand bath under a stream of dry nitrogen. When all odor of trifluoroacetate was gone, 50 µl n-butanol was added. After 15 minutes an ethereal solution of diazobutane was added with gentle swirling until the yellow color persisted. After 10-15 minutes a 5% solution of acetic acid in benzene was added until the color disappeared. The samples were then evaporated to the desired volume in a sand bath under dry nitrogen.

2) Preparation of diazobutane.

Due to the sensitive nature of diazo compounds, the preparation of diazobutane was performed only with polished glassware. In a 125 ml Erlenmeyer flask cooled in an ice bath 2.3 g KOH was dissolved in 2.3 ml of water; 50 ml diethyl ether (ACS reagent) was added and the mixture cooled to 0° C. Then, via a powder funnel, 1.89 g (10 mM) of N-butyl-N'-nitro-N-nitrosoguanidine was added in 3-4 portions with vigorous swirling after each addition. The mixture was

allowed to stand for 10-15 minutes in an ice bath, and then the ether layer was decanted into a 25 x 190 mm test tube. The tube was fitted with a serum cap, placed in a dry ice-acetone bath (-78° C) and a 20 ml syringe containing Drierite was attached to release the partial vacuum formed. The reaction mixture was swirled each 15-20 minutes for two hours, then decanted into another cold test tube, leaving behind most of the precipitate. The mixture was then allowed to stand for another hour in the dry ice-acetone bath with swirling each 15 minutes. The final preparation was decanted into a test tube held in an ice bath.

3) Isolation of CP 50435-^{14}C from rat urine.

A cation-exchange column was prepared by pouring an aqueous slurry of AG-50W-X8 (H^+ form; 200/400 m) (Bio-Rad Laboratories, Inc., Richmond, Calif.) into a 2.4 x 60 cm Glenco glass column fitted with a teflon Luer tip and filter. The final resin bed was 2.4 x 58 cm. The resin was washed with distilled water until the eluate was colorless. A 12 or 24 hour urine sample which had been diluted to 25-35 ml with distilled water was loaded onto this cation exchanger. The column was then eluted with 1500 ml distilled water and 20 ml fractions were collected using an LKB 7000 fraction collector.

The eluent fractions from the cation-exchange chromatography which contained radioactivity were pooled and loaded onto an anion-exchanger of AG-1-X8 (HCO_3^- form, 200/400 m) (Bio-Rad Laboratories, Inc., Richmond, Calif.). The resin was prepared from the Cl^- form by passing 1M NH_4HCO_3 (2L 1M NH_4HCO_3/lb resin) through the resin. The resin was then washed with water until the eluate was neutral. An amount of resin equivalent to 20 g dry weight was slurried with water and poured into a Pyrex glass column forming a resin bed of 1.5 x 15 cm. The sample was then applied in a volume of 200 ml. The column was eluted with 200 ml distilled water, followed by 300 ml 0.2N NH_4HCO_3. Fractions of 5 or 10 ml were collected.

The eluent fractions from the anion exchanger which contained ^{14}C-activity were pooled and reduced to 1-1.5 ml and loaded onto a Bio-Gel P-2 (Bio-Rad Laboratories, Richmond, Calif.) column for gel-filtration chromatography. The gel was prepared by suspension in 0.5N acetic acid, deaeration with a water aspirator and pouring the slurry into a 1 x 110 cm Glenco column to form a bed of 1 x 108 cm.

After the sample was charged onto the column, the sample flask was rinsed with 1-2 ml distilled water which was added to the column. The column was then eluted with 200 ml 0.5N acetic acid and 1-2 ml fractions collected.

4) Thin-Layer Chromatography of the purified fractions.

The Bio-Gel fractions which contained ^{14}C-activity were pooled, reduced to small volume and an aliquot applied to a 20 x 20 cm thin-layer chromatography (TLC) plate with a 250 μ layer of microcrystalline cellulose (Quantum Q-2, Quantum Industries, Fairfield, N. J.). The plates were developed first in a phenol-water system of the following composition:

90% Phenol (Fisher Certified)	84 ml
Distilled Water	16 ml
Glacial Acetic Acid	1 ml
EDTA	37.2 mg

After the plates were air-dried they were rotated 90° and developed in a modified semi-stench solvent system (12) of the following composition:

EDTA	1.2 g
17N NH_4OH	100 ml
Distilled Water	475 ml
1-Propanol	350 ml
2-Propanol	75 ml
1-Butanol	75 ml
iso-Butyric Acid	2500 ml

(mix and let stand 24 hours before use)

Colorimetric visualization of the TLC plates was accomplished by spraying with ninhydrin (Quantum 1130 or Gelman 72818) or with a modified Hanes reagent (13, 14). ^{14}C-Activity on the TLC plates was detected by means of the Beta Camera, Model 6000 (Baird-Atomic, Inc., Middlesex, Mass.). Permanent copy of the Beta Camera CRT image was reproduced by means of a Polaroid Pack Camera with Type 107 film.

5) Nuclear Magnetic Resonance (^1H-NMR) of the isolated fractions.

The samples were lyophilized in 3.5 ml vials (Swharz-Mann No. 915) and exchanged twice with 99.8% deuterium oxide (D_2O) (Mallinckrodt Chem. Co., St. Louis), lyophilizing the sample after each exchange step. The sample was then dissolved

in 0.1 ml 100% D_2O (Diaprep Div., Aldrich Chem. Co.) and transferred to a 0.3 ml vial (Reacti-Vial, Pierce Chemical Co., Rockford, Ill., No. 13220). The original vial was washed with an additional 0.1 ml 100% D_2O which was added to the sample and the entire sample was lyophilized.

Immediately before running the spectra, the samples were dissolved in 10 µl 100% D_2O and the solution filtered through a Flath-Ludin syringe filter (Hamilton Co., Reno, Nevada, Cat. No. 76500) directly into a capillary tube. An additional 5 µl D_2O was used to rinse the vial and was also filtered directly into the capillary tube. The capillary was inserted into a teflon chuck (Wilmad, Buena, N. J., No. 529-B) which was then placed in a 7 in. NMR tube (Wilmad, No. 529-PP).

High resolution proton spectra (60 mHz) were run on a Varian T-60 and/or a JEOL JNM C-60-HL spectrometer. The latter was equipped with a JNM-AS-1 resolution stabilizer, JRA-1 spectrum accumulator, Monsanto 100-A frequency counter, an external Hewlett Packard Model 200 CD wide range audio oscillator, a Hewlett Packard Model 5245L electronic counter, a hetero spin decoupler (JNM-5D-HC) and an RF oscillator adapter (JNM-OA-1).

All ^{1}H-NMR spectra were calibrated using HOD as the internal reference and were decoupled using the hetero spin decoupler (JNM-5D-HC) and an RF oscillator adapter (JNM-OA-1).

6) <u>Gas-Liquid Chromatography of the isolated fractions</u>.

Following NMR analysis the samples were derivatized as described for the analytical standards and were examined by gas-liquid chromatography (GLC) on a Perkin-Elmer Model 900 which is a dual column instrument equipped with thermal conductivity (TC), flame ionization (FID) and phosphorus specific (FPD) detectors. The samples were analyzed on a 6 ft x 4 mm (i.d.) glass column packed with 1.5% OV-17 on Chromosorb W-HP (80/100 m) programed from 120-240° C at 10°/min.

7) <u>Mass Spectrometry of the isolated fractions</u>.

The final analysis of the purified fractions was performed by coupled gas chromatography and mass spectrometry. A PE-900 GLC was coupled through a Bieman separator to a Perkin-Elmer Model 270 mass spectrometer (MS) operating at 70 ev in the GLC mode. A Honeywell 2106 Visicorder

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