UNITED STATES ENVIRONMENTAL PROTECTION AGENCY

0614
TOX REV 275

DATE: October 4, 1977

SUBJECT: N-nitrosoglyphosate, report on toxicologic data on it for the record, including evaluation as support for human safety of glyphosate.

FROM: TB/RD

TO: Mr. R. Taylor, PM

PP Nos. 5F1560; 6F1733 & 6H5115; 6F1758 & 6H5126; 6F1798; 6E1809; 6F1861; 6H5144; 7F1892; 7F1904

Monsanto
St. Louis, Mo. 63166

Petitioner submits studies on N-nitrosoglyphosate in support of the above-named PP's. We have abstracted them in the "Appendix," to this memo. Exact title of the volume containing the studies is given in the appendix, p. 1.

SUMMARY:

1. Part A (oral LD_{50} of N-nitrosoglyphosate determined in rat), from Younger Laboratory, is judged satisfactory.

2. Part B (Ames-type in vitro test for mutagenicity of N-nitroso-glyphosate on micro-organisms), from Litton Bionetics, is judged not acceptable, pending satisfactory answers to these questions: (A) What are actual amounts of N-nitrosoglyphosate and of positive control chemicals which were used in the test, which are expressed as "microliters" (per plate)? (B) Was a sufficiently large amount of N-nitrosoglyphosate tested so that, even if it were of considerably less mutagenic potency than the positive control chemicals used, it could have yielded a positive result? (C) What assurance is there that N-nitrosoglyphosate was stable in this test system?

3. Part C (mouse dominant-lethal mutagenicity test on N-nitroso-glyphosate), from Industrial Bio-test, is judged unacceptable because of the very low (intraperitoneal) doses of N-nitrosoglyphosate used (5 and 10 mg/kg), relative to the rat oral LD_{50} of N-nitrosoglyphosate (5-7,000 mg/kg), unless Petitioner can justify properly use of this low doseage.

4. Part D (teratologic test on N-nitrosoglyphosate in rabbit), from Industrial Bio-test, is judged unacceptable. If repeated, it should use larger test doses, some of which are demonstrated to be toxic to the maternal rabbit; use at least three dose-levels; and, preferably, include a positive control.

5. Part E (Bio/dynamics Inc.) provides a 6-month status report of a projected 18-month carcinogenicity study on N-nitrosoglyphosate in the hamster. No unusual effects (except for hign incidence of eye abnormalities in animals from all groups) are reported to date.

6. Use of these data by TB, in regard to certain tolerance and registration requests (for glyphosate), is noted briefly in this memo (p. 3).

EVALUATION:

Following is the evaluation of data on N-nitrosoglyphosate, which comprise Parts A-E, inclusive, of the volume, title of which is given at top of APPENDIX (p. 1) to this memo.

Data in Part A (Younger Labs.) are judged satisfactory. They show oral LD_{50} of N-nitrosoglyphosate (rat, M and F) to be, variously, 7.60 g/kg BW (95% CF's of 7.07-8.21 g/kg) and 4.35 g/kg BW (95% CF's of 4.09-4.61).

Data in Part B (Litton Bionetics), which show N-nitrosoglyphosate as negative for mutagenicity, tested in vitro in Ames-type test both with and without metabolic activation, are not judged acceptable until three questions are satisfactorily answered.

1) What are the actual amounts of test compound (N-nitrosoglyphosa and of positive control chemicals which were used in the test, which are expressed as "microliters" per plate? (Microliter is a measure of volume, not weight; if used (microliter), it should be accompanied by specific gravity of the chemical or, if a solution is used, the concentration of the solution.)

2) What is the rationale for choice of the respective amounts of N-nitrosoglyphosate and positive control chemicals? I.e., was a sufficiently large concentration of N-nitrosoglyphosate tested so that, even if it were weaker with respect to mutagenicity than, say, positive-control chemical, methylnitrosoguanidine, it could still have given a positive response?

3) What assurance is there that the test chemical (N-nitrosoglyphosate) was stable in the test system used?

Data in Part C (Industrial Bio-test Labs.), which show negative activity for N-nitrosoglyphosate in the mouse dominant-lethal mutagenicity test, at single ip doses of 5 and 10 mg/kg body weight, are not judged adequate unless choice of these doses is justified. I.e., when a dose approaching a maximum tolerated dose is ordinarily used in this test, why was such a low one (10 mg/kg) relative to the rat oral LD_{50} (five to seven thousand mg/kg) used?

Data in Part D (Industrial Bio-test Labs) are not judged adequate, in claiming to show that N-nitrosoglyphosate is not a teratogen in the rabbit, for the following reason

The doses of N-nitrosoglyphosate used are judged too low; the top dose used (30 mg/kg BW per os) is less than 1% of the rat LD_{50} for N-nitrosoglyphosate; whereas an appreciable fraction of the maternal LD_{50} is generally employed. Nor was there evidence of maternal toxicity; since the three deaths (maternal) in the top-dose group are not ascribed to the test compound by the experimenters, and there was no difference in body weight between test and control groups.

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Evaluation (continued):

The study should be repeated, using larger test doses which are shown to be toxic to mothers, at least three dose-levels, and, preferably, a positive control.

Data in Part E (Bio/dynamics Inc.) consist of a six-month "status report" of a projected 18-month carcinogenicity study in the hamster. To date, no outstanding effects are reported. We note, there is a high incidence of eye abnormalities in hamsters of all groups. We question whether some aspect of animal husbandry in this study could contribute to the findings, e.g, spread of infection; since all animals with eye abnormalities pre-test were eliminated.

Recent actions with respect to requested glyphosate tolerances (and registration actions.

We note here that, recently, TB has recommended for several requested temporary tolerances for glyphosate, following CB evaluation of probable content of N-nitrosoglyphosate in glyphosate-treated commodities. We also recommended for an EUP (524-EUP-24) on 9/12/77, based, in part, on our evaluation of some of the data in the present submission.

However, we have not recommended, and do not intend to recommend, for either permanent glyphosate tolerances or registrations, based on these data.

M. Q., 10/4/77

Mary L. Quaife, Ph.D., TB/RD
October 4, 1977

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E 1/9/78

Glyphosate, Roundup®, EPA Reg. No. 524-308, N-phosphonomethylglycine, Special Report #478, 5/6/77, Monsanto Research Dept., Accession No. 229785, Vol. 1 of 2, "Toxicology, Crop Residue and Metabolism Studies of N-(Phosphonomethyl)-glycine: N-Nitrosoglyphosate," Sec. C, Toxicology.

Pt A. Acute oral LD_{50} of 20% Aq. solution of CP76100 (CP 76976, N-nitroso-glyphosate - Sec. C, first page, this report), Younger Labs., St. Louis, Mo.

Test of 4/19/76, Project No. Y-76-122.

A sample identified as Lot No. 226776 of above substance, 20% aq. soln was given as a single oral dose at levels of 31.6, 39.8, or 50.1 g/kg body weight to groups of 5 young adult rats (Sprague-Dawley) of mixed sexes to determine the LD_{50}.

Results. Rats which died did so within 2 days. Toxic signs shown were reduced appetite and activity, increasing weakness, collapse, and death. Decedents at autopsy showed, grossly, lung hyperemia, slight liver discoloration, and gastrointestinal inflammation. Viscera of survivors (14 days) appeared normal.

The oral LD_{50} of the test solution is 38.0 g/kg with 95% CF's of 35.3 - 41.0 g/kg BW.

The oral LD_{50} of the active ingredient, N-nitrosoglyphosate, is 7.60 g/kg BW with 95% CF's of 7.07 - 8.21 g/kg.

Test of 12/12/75, Project Y-75-309.

A sample identified as Lot No. Ag 226763-2 thru 5, was given in single oral dose to groups of 5 ca. 200-g Sprague-Dawley rats (mixed sexes) each at 2.51, 3.16, 3.98, or 5.01 g/kg BW of active ingredient in order to determine the oral LD_{50}. (Given as 20% aq. solution.)

Results. Time of death, signs of intoxication, and gross autopsy findings of decedents and survivors were as described for the 4/19/76 test (above).

The oral LD_{50} of active ingredient, N-nitrosoglyphosate, is 4.35 g/kg BW for the (M + F) rat(s). Confidence limits (95%) are 4.09 - 4.61 g/kg BW.

Pt. B. Mutagenicity evaluation of BIO-76-116; CP-76100, Lot 5-701, final report, Litton Bionetic, Inc. Kensington, Md, Proj. No. 2547, 6/22/76.

The test compound identified in the title, a clear liquid, received 5/24/76) was examined in a series of *in vitro* microbial assays using *Salmonella* and *Saccharomyces* indicator organisms, both directly and with liver microsomal enzyme preparations from Aroclor-induced rats. Concentrations used are said to span dose that did not show a toxic effect and dose that gave evidence of some chemically-induced physiologic effect.

Results. These are presented in a table on the next page.

4. SUMMARY OF PLATE TEST RESULTS

A. Name or code designation of the test compound: BIO-76-116; CP-76100, Lot T-701

B. Solvent: DMSO

C. Test date: May 26, 1976

D. Concentrations of the test compound: (1) 0.01 µl (2) 0.1 µl (3) 1 µl (4) 5 µl/plate

TEST	SPECIES	TISSUE	REVERTANTS PER PLATE TA-1535	TA-1537	TA-1538	TA-98	TA-100	D4*
NONACTIVATION								
Solvent control	---	---	64 7	19	23	33	155	50
Positive control**	---	---	>10³--	>10³	>10³	483	319	101
Test compound (1)	---	---	82 --	13	24	38	127	49
(2)	---	---	96 --	24	30	34	139	48
(3)	---	---	107 7	8	35	37	168	50
(4)	---	---	122 7	14	23	65	137	44
ACTIVATION								
Solvent Control	Rat	Liver	29	15	20	229	142	35
Positive control***	Rat	Liver	230	300	500	>10³	315	44
Test compound (1)	Rat	Liver	76	12	20	209	142	37
(2)	Rat	Liver	28	15	18	201	131	39
(3)	Rat	Liver	20	8	21	224	135	34
(4)	Rat	Liver	19	4	15	189	137	35

* Try^+ convertants per plate

** TA-1535 MNNG 10 µl/plate
TA-1537 QM 10 µl/plate
TA-1538 NF 100 µg/plate
TA-98 NF 100 µg/plate
TA-100 MNNG 10 µl/plate
D4 MNNG 10 µl/plate

*** TA-1535 ANTH 100 µg/plate
TA-1537 AMQ 100 µg/plate
TA-1538 AAF 100 µg/plate
TA-98 AAF 100 µg/plate
TA-100 ANTH 100 µg/plate
D4 DMNA 100 µmoles/plate

APPENDIX p. 2

Litton mutagen test, Pt. B (continued).

Non-activation test results. These were all negative. Doses 3 and 4 with strain TA-1535 were repeated because of slightly increased mutant frequencies. The repeat test was negative.

Activation test results. Results of tests carried out on N-nitrosoglyphosate in presence of the rat liver activation system were all negative.

Conclusion: N-nitrosoglyphosate, as tested here, did not demonstrate mutagenic activity in any of these assays. (It is noted that this report (p. 3) states that, "Aliphatic nitrosamines will give positive results using this modification (of the Ames test).")

Addendum: The positive control chemicals used in the assay are, for nonactivation, methylnitrosoguanidine (MNNG), 2-nitrofluorene (NF), and quinacrine mustard (QM). For activation, they are 2-anthramine (ANTH), 2-acetylaminofluorene (AAF), 8-aminoquinoline (AMQ), and dimethylnitrosamine (DMNA).

Pt. C. Dominant-lethal study with CP 76100 in albino mice, Industrial Bio-test Labs., Inc., Northbrook, Ill., BTL-76-31, IBT No. 8533-08920, 1/4/77.

A dominant-lethal mutagenicity study was conducted on albino mice of the Charles River strain (C. R. Breeding Labs., Wilmington, Mass.) on CP 76100 (19.8% active, Lot No. T-701, Ref. No. 76-35). Groups of 12 male mice each received a single ip injection of either 5 or 10 mg CP 76100 (N-nitrosoglyphosate) per kg body weight. A control group treated with 1 mg NaCl/kg BW was included. Following adminstration of test or control chemical, the males were mated weekly with 3 untreated virgin females for 6 consecutive weeks.

Results. No treated or control males died. Male and female fertility indices compared well among test and control animals. Mutagenicity data, summarized in table on next page, whether judged as percent of early deaths or by comparing numbers of live embryos per female, showed comparable results for test and control males and, also, with cumulative control values.

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* Pre-implantation loss = $\frac{\text{No. of corpora lutea} - \text{No. of implant. sites}}{\text{No. of corpora lutea}} \times 100$

$\frac{\text{Number of early resorption sites}}{\text{Number of implantation sites}} \times 100$ (See A in table).

$\frac{\text{Embryos/female control} - \text{embryos/female test}}{\text{Embryos/female control}} \times 100$ (See B in table).

Values for each group were obtained by comparing the mean values of that group to the values of the contemporary control group (a in table) and to cumulative control data (b in table).

Attachment p. 4

TEST MATERIAL: CP 761.

Dominant Lethal Study - Albino Mice

Mutagenic Data

Group	Dose Level (mg/kg)	Week No.	Pre-Implantation Loss	Mutation Rate A	Mutation Rate B a	Mutation Rate B b
TC	None*	1	16.0	6.3	-	8.7
		2	12.6	6.9	-	5.3
		3	16.3	8.0	-	11.3
		4	20.1	3.3	-	10.4
		5	14.5	4.7	-	6.9
		6	8.5	3.1	-	-1.7
T-I	5	1	10.6	5.0	-4.8	4.3
		2	11.2	5.9	-3.7	1.8
		3	14.2	3.6	-8.8	3.5
		4	10.3	5.9	-7.8	3.5
		5	16.6	7.8	5.6	12.1
		6	7.1	5.8	0.0	-1.7
T-II	.10	1	6.7	7.2	-10.5	-0.9
		2	8.6	7.8	-3.7	1.8
		3	10.8	5.4	-9.8	2.6
		4	5.9	4.6	-16.5	-4.3
		5	18.9	2.9	3.7	10.3
		6	8.0	6.2	2.5	0.9

* Control males were treated with 1.0 mg/kg NaCl.

Mouse dominant-lethal test (Pt. C) continued.

Conclusion: As tested in this system, N-nitrosoglyphosate did not show evidence of being a mutagen.

Addendum: Principle of this test method is as follows: A mutation is a change in the character of a gene such that morphologic, physiologic and/or biochemical alterations are produced. If this change in gene character occurs in the germinal cell, the alteration can be transmitted to succeeding generations. Changes of this nature can be artificially induced (irradiation, chemical exposure) or they may be spontaneous. A dominant-lethal mutation occurring in a male germinal cell may lead upon fertilization by the affected cell, to the failure of development of the resulting zygote beyond the blastocyst stage (implantation). Male mice, treated with the test compound, are mated with untreate females. The numbers of pre-implantation losses and early resopptions (deciduomata) in female mice, dissected at mid-gestation, are used to calculate the mutation rate based on the induction of dominant-lethal mutations.

Pt. D. "Teratogenic study with CP 76100-2 in albino rabbits," BTL-76-32, 1/4/77, IBT No. 8580-08921, Industrial Bio-Test Labs., Inc., Northbrook, Illinois.

A sample, identified as CP 76100-2, Lot Number T-701, was administered to groups of 17 artificially inseminated New Zealand albino rabbit does at 10 or 30 mg/kg body weight on gestation days 6 through 18, inclusive. A negative control group - 17 does - each received 3 mg/kg BW/day. No positive control group was included in the study. All females were killed on day 29.

Results. Numbers of resorption sites per 100 implantation sites were 13.3, 15.7, and 36.8 for control, 10-mg/kg, and 30 mg/kg groups respectively. This is reported to show that the top dose caused embryo toxicity.

Other aspects of reproduction shown in this study are tabulated on the next page. (Test substance did not affect body weight of pregnant females.) Two control and no test does aborted. Due to this, the controls had significantly less live young per 100 implantation sites than the 10-mg/kg group; however, they showed no increase in number of resorption sites per 100 implantation sites. As noted, the 30-mg/kg group had notably more resorption sites per 100 implantation sites; they also had notably fewer live young per 100 implantation sites. (Twenty-hour survival of progeny was 89.5, 83.2, and 78.5% for control, low- and high-dose groups.) (Three 30-mg/kg does died during the test of undetermined causes.

Two (1.8%) 10-mg/kg fetuses and one (1.3%) 30-mg/kg fetus had unilateral talipomanus (clubhand), compared to no controls. Control incidence of external fetal abnormalities in these animals in this laborato is said to be 0.58% (range of 0 to 5%). No gross internal abnormalities in the fetuses were found. Of skeletal abnormalities (shown on clearing and alizarin staining), control fetuses had none; two low-dose fetuses (1.8%) had mal-formed parietal bones; and one high-dose fetus (1.3%) had dual ossification of sternum section(s); controls are said to have shown these in previous studies in this laboratory.

TABLE III

TEST MATERIAL: CP 76100-2

Teratogenic Study - Albino Rabbits

Summary of Reproductive Effects

Group	Test Material	Dose Level (mg/kg/day)	Number of Pregnant Animals	Number of Implantation Sites	Number of Resorption Sites Early	Number of Resorption Sites Late	Number of Does Showing Resorptions	Number of Live Young	Number of Resorption Sites per 100 IS	Number of Live Young per 100 IS	Number of Fetuses Aborted	Number of Does Showing Abortions
TC	Sodium ion(Na^+)	3.0	13	120	12[a]	4	6	86	13.3	71.7	18	2
T-I	CP 76100-2	10.0	14	134	8	13	9	113	15.7	84.3	0	0
T-II	CP 76100-2	30.0	13	125	30[b]	16[c]	9	79	36.8	63.2	0	0

IS-Implantation Sites

a One control doe accounted for 9 of the 12 early resorption sites.
b Two T-II does accounted for 16 of the 30 early resorption sites.
c One T-II doe accounted for 9 of the 16 late resorption sites.

Incidence of so-called "incidental skeletal findings" (incompletely ossified sternum sections, non-ossified sternum sections, and supernumerary ribs) is 89.5, 80.5, and 82.2% for control, 10-mg/k , and 30-mg/kg fetuses, respectively.

Conclusion: Taking results at face value, one would conclude that N-nitrosoglyphosate is not shown to be a teratogen in this test. However, we have reservations about this conclusion and discuss them in our evaluation portion of this memo.

Pt. E. Proj. No. 76-1401, BDN-76-36, "An eighteen-month oral toxicity study on CP 76100 in hamsters," status report, Bio-dynamics Inc., status as of 11/6/76.

Groups of 70 M and 70 F hamsters (strain not given) each receive either 0, 3, 10, or 30 mg/kg BW/day of a sample, identified as CP 76100, in a carcinogenicity study on N-nitrosoglyphosate, designed to last 18 months. Material was given (route not specified, presumably, per os) as 10 ml/kg body weight/day of a solution (solvent not specified) of 19.8% active ingredient by weight. Controls received NaCl, 7.6 mg/kg BW/d

Animals which died in first two months of study were replaced, and five more hamsters/group/sex were added at two months.

Animals were observed for behavior and any deaths, daily, and for tissue masses, weekly. They were weighed and food consumption determined either weekly or biweekly. The schedule for laboratory studies, to be done on 10/sex/group, is: 45 days, and 3, 6, 12, and 18 months. These include: Hemoglobin, hematocrit, RBC, total and differentia WBC, RBC morphology, and clotting time determinations and Serum glutamic-pyruvic transaminase, alkaline phosphatase, blood urea nitrogen, and fasti glucose assays and Urinalysis for appearance, protein, glucose, pH, specif gravity, ketones, bilirubin, and occult blood.

Animals which died or were killed in moribund state were to be autopsied, as were 10/sex/group at 6 and 12 months and all survivors at 18 months (further details not given).

Ophthalmoscopic examinations were to be made, pretest, and at 6, 12, and 18 months.

Results. Except for some higher S-GPT values in females at 3 months (1 control female, and mean values for 10- and 30-mg/kg animals), the only unusual finding by six months on test was the very high occurrence of eye abnormalities in hamsters from all groups. Seventy hamsters out of ca. 540 survivors are listed as having eye abnormalities a the 6-month examination by L. F. Rubin, VMD. According to the report, p. all animals with eye abnormalities (eleven) seen pre-test were removed from the stu thus the eye findings developed after start of the study. Ca. one-third o the lesions are listed as inflammatory, with most of these in both eyes.

To date, one male in the mid-dose group was noted to have a mass at weeks 20 and 21.