6-14-78
Caswell No. 661A

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY

DATE: June 14, 1978

TOX R 000271

SUBJECT: N-nitrosoglyphosate, TOX studies, Accession No. 229785, submitted by Monsanto, 5/6/77 (RD 131, Special Rept. 478" L. H. Hannah et al.): TB evaluation of Petitioner's (3/20 and 5/24/78) comments on EPA (2/13/78 and 4/20/78) letters concerning them - glyphosate PP 5F1560 (and other glyphosate PP's)

FROM: TB/RD, Mary L. Quaife, Ph.D. MLQ 6/15/78

TO: Mr. R. Taylor, PM 25 7/28/78

PP No. 5F1560
(Reg. No. 524-308)

Monsanto
St. Louis, Missouri

CONCLUSIONS:

1. Results of the Ames-type in vitro test, with and without metabolic activation, as conducted, are negative for mutagenicity of N-nitrosoglyphosate.
   Classification: Core-minimum
   a. Levels of N-nitrosoglyphosate tested are judged lower than optimal.

2. Results of the mouse dominant-lethal test, as conducted, are negative for mutagenicity of N-nitrosoglyphosate. Test done at IBT.
   Classification: Core-minimum
   a. Test doses used are too low, relative to the oral acute toxicity (rat).

3. Results of rabbit teratologic test, as conducted, are negative for teratogenicity of N-nitrosoglyphosate. Test done at IBT.
   Classification: Supplementary
   a. Too low test doses used, relative to (rat) acute oral toxicity.
   b. Too few dose levels included.
   c. Positive control omitted
   d. Statistical analysis (Mann-Whitney) shows the observed differences between 30-mg/kg and control groups in numbers of resorptions and numbers of live young/100 implantation sites for each litter can be ascribed to chance, not to toxicity as Petitioner alleges.

RECOMMENDATION: We recommend that this TB evaluation of studies on N-nitrosoglyphosate be added to the record for TOX data on glyphosate, (PP No. 1560 and others).

N.B. Petitioner included a 6-month interim report of carcinogenic study on N-nitrosoglyphosate in the hamster. Since the report was dated May, 1977, the projected 18-month study should be completed, or nearly so.

added by Junk to this copy 9/15/78, MLQ.

EPA FORM 1320-6 (REV. 3-76)

Following are excerpts from our TB memos [which correspond to letters (memo title, above)]; Petitioner's reply; and our comment/conclusion:

Regarding Ames-type in vitro test for mutagenicity of nitroglyphosate (using microorganisms) from Litton Bionetics, LBI Project No. 2574,

"2(A) What are actual amounts of N-nitrosoglyphosate and of positive control chemicals used in the test....(per plate)?"

Petitioner's answer. Amounts of 0.2, 2, 20, and 100 micrograms (µg) per plate were used.

Amounts of positive controls used are - methylnitrosoguanidine, 10 µg/plate; quinacrine mustard, 2-nitrofluorene, 2-anthramine, 2-acetylaminofluorene, and 8-aminoquinoline, each 100 ug/plate; and dimethylnitrosamine, 7,400 µg/plate.

Our coment/conclusion on 2(A). This factual answer is satisfactory.

"2(B) Was a sufficiently large amount of N-nitrosoglyphosate tested such that, even if it were of considerably less mutagenic potency than the positive control chemicals used, it could have yielded a positive result?"

Petitioner's answer. "Highest dose of nitrosoglyphosate tested was such that a slight degree of toxicity is evident....(Petitioner) did not perform toxicity tests on nitrosoglyphosate....(However), slight toxicity in the Ames test results is shown....with strain TA-1537 at the high dose in activation assay, as evidenced by the reduced number of revertants...."

".....Positive controls produced significant mutagenic responses at similar or substantially lower (amounts) than that of N-nitrosoglyphosate used. Thus, a sufficiently large amount was used."

Our comment on 2(B). We do not find the rather small decrease - in absolute numbers - of revertant colonies (from 15 to 4, cf. Table 1) especially convincing evidence of toxicity caused by the top dose (100 µg) of nitrosoglyphosate tested; it could been due to spontaneous variation. Such "toxicity" was demonstrated in only one of 12 test systems used (six, each with and without liver S-9 mix present).

We note, in Ames paper (supplied by Mr. S. Biscardi of TB) - Mutation Res. 31, 347-64 (1975), that Ames requires a negative result at a level of test chemical equal to 500 µg/plate before considering it negative for mutagenicity; whereas top level of nitrosoglyphosate used in present test is 100 µg/plate. Thus, Petitioner's highest level of test compound is only one-fifth that recommended by Ames.

Whereas positive controls were used in amounts equal to, or one-tenth of, the amount of test compound, yet in one test system, the positive control, N-dimethylnitrosamine, was used at 7,400 ug/plate vs. no more than 100 µg of nitrosoglyphosate. Again, it appears that - especially in this latter test system - the amount of nitrosoglyphosate tested is unduly low to show a response if is a weak mutagen.

Our conclusion on 2(B). We do not consider the levels of nitrosoglyphosate that were tested are optimal to show positive results, if it is a weak mutagen.

"2(C) What assurance is there that N-nitrosoglyphosate was stable in the test system?"

Petitioner's answer. Petitioner believes that it is as stable as the positive control, methylnitrosoguanidine.

Our comment/conclusion on 2(c). We do not require further information/comment on this point.

General conclusion on 2. The Ames test, as conducted, is negative for mutagenicity of N-nitrosoglyphosate.

Classification (Ames test on nitrosoglyphosate): Core-minimum.

1. Top level tested is only one-fifth that recommended by Ames.
2. Evidence that this top level was limiting due to its toxicity to the microorganisms is considered to be weak.
3. Indeed, in one test system, the positive control, dimethylnitrosamine, was used at a level 74 times the amount of test compound used.

For the above reasons, the levels tested are judged below optimal.

Regarding mouse (dominant-lethal) test on nitrosoglyphosate from Industrial Biotest,

"3(C) Part C (mouse dominant-lethal mutagenicity test on N-nitrosoglyphosate) from Industrial Biotest is judged unacceptable because of the very low intraperitoneal (ip) doses of N-nitrosoglyphosate used (5 and 10 mg/kg, respectively) relative to its high oral LD50 (ca. 5,000 to 7,000 mg/kg) in the rat - unless the low doses can be justified."

Petitioner's reply. The choice of dose levels (5 and 10 mg/kg) was to provide amounts far in excess of any intended exposure to N-nitrosoglyphosate. They should be accepted for regulatory purposes. (In reply to a further question (whether an intraperitoneal (ip) LD50 for nitrosoglyphosate in the mouse is known), Petitioner says he knows of none.)

Our comment on 3(C). At least, the ip LD50 in the mouse should have been determined and the test doses chosen to be substantial fractions of that. However, since an ip LD50 is, usually, considerably lower than an oral LD50, we now judge this study acceptable.

We disagree sharply with Petitioner that doses should be chosen based on probable human exposure. They should be in the range of dosage that produces some definite toxic effect.

Our conclusion on 3(C). Test as conducted is negative for mutagenicity

Classification (dominant-lethal on nitrosoglyphosate): Core-minimum

1. Test doses used are too low, relative to the oral acute toxicity (rat).

Regarding rabbit teratologic test on nitrosoglyphosate from IBT,

"4 Part D (teratologic test by Industrial Biotest on N-nitrosoglyphosate in the rabbit) 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."

Petitioner's reply. "....Study (had) adequate dosage levels, number of dose levels, and with a strain of rabbit of known sensitivity to teratogens....(There is) a significant increase in the number of resorptions and decreased number of live young/100 implantation sites in high-dose group...indicative of maternal and/or fetal toxicity.... which is, therefore, the maximum acceptable dose level....(One uses) a third dosage only when a teratogenic effect is shown on high-dose group (which is) not the case here."

Our comment on 4. We do not agree with Petitioner's reply.

1. We reiterate, experimental design is deficient, due to too few dose levels; too low doses relative to (rat) oral LD50; and omission of positive-control group.

In "Current Methodology in Teratology Research," Collins (in,"New Concepts in Safety Evaluation," edited by M. Mehlman et al , John Wiley and Sons, New York, Chap. 6, pp. 155-75) outlines the teratology protocol (rat) used for testing the GRAS (generally recognized as safe) substances by FDA: At least 20 pregnant females/group to be used;
At least four dose levels to be included;
The high dose to be 10% of maternal oral LD50, if LD50 < 16 g/kg;
The low dose to be 1% of the LD50;
Two logarithmically spaced, intermediate groups to be used; and
Positive and negative controls to be included.

In contrast, Petitioner's study (rabbit) used two dose levels, the higher being less than 1% of the oral LD50 (rat), and the lower, only one-third of the higher dose, and no positive control. Pregnant females included per dose level number 13 to 14. A negative control is included.

2. We do not agree that Petitioner's higher dose used caused maternal and/or fetal toxicity and, thus, qualifies as a "maximum acceptable' dose level.

Petitioner bases this claim on, "....(the)....revealed....significant increase in the number of live young/100 implantation sites in the.... 30-mg/kg (higher-level) nitrosoglyphosate group." He says, "higher dosage levels would be expected to severely reduce the number of viable young available to show terata," (3/20/78 letter, F. R. Johannsen, Monsanto - PM R. J. Taylor, EPA).

The letter does not indicate to what dosage group the above 30-mg/kg group values are compared; one might assume it is to the controls. However, inspection of the data does not clearly reveal such biologically significant differences, as are claimed, between top dose (30-mg/kg) group and controls.

We have made statistical comparisons between control and high-dose (30-mg/kg) groups (courtesy of Dr. R. Schmitt, TB).

Statistically, by one-tail Fisher's exact test, the crude ratio, number of resorptions/100 implantations sites, is significantly ($p < 0.001$) higher for the 30-mg/kg rats than for controls. This would correspond to Petitioner's claim (above) of a "significant increase."

However, by the non-parametric Mann-Whitney test (used for teratologic evaluation, as noted in, "Teratogenicity of Pesticides," Chap. 8, pp. 655-77, in the "DHEW Secretary's Commission on Pesticides and Their Relationship to Environmental Health, December, 1979), the "null hypothesis" is not refuted, and the difference between the 30-mg/kg and control groups in numbers of resorptions/100 implantation sites for each litter can be ascribed to chance.

Furthermore, use of these two statistical technics showed no significant difference between control and 30-mg/kg groups with respect to the second data category. That is, there is no significant ($p > 0.05$) difference in the crude ratio, number of live young/100 implantation sites, between control and 30-mg/kg groups by Fisher's one-tail test. And the differences in numbers of live young/100 implantation sites for each litter between 30-mg/kg and control groups can be ascribed to chance by the Mann-Whitney test.

See attached pages (5 and 7) for detailed calculations.

Our conclusion on 4. Results of the (rabbit) test as conducted are negative for teratogenicity of nitrosoglyphosate.

Classification (IBT rabbit teratologic test on nitrosoglyphosate): Supplementary

1. Inadequate experimental design - doses used too low, relative to (rat) oral LD50; too few dose levels included; and positive control omitted.

Note: Our statistical (by Mann-Whitney) analyses do not uphold Petitioner's contention that top dose, 30 mg/kg, is demonstrably a maximum acceptable dose; since the observed increase in numbers of resorptions and decrease in numbers of live young/100 implantations sites in 30-mg/kg group, compared to controls, can be ascribed to chance.

PP NO. 5F1560/6/14/78
Mann-Whitney test
Resorptions/100 Implantation Sites

Control vs. 30-mg/kg nitrosoglyphosate g

30 mg/kg

RESP / 100 IMP

CONTROL RESP / 100 IMP for each later

0.11
0.73
2.73
0.20
0.56
5.00
0.45
0.20
0.67
0.00
0.00
0.00
1.00

N 13.00
MEAN 0.36
MEDIAN 0.20
MIDRANGE 0.50
VARIANCE 0.12
STD. DEV. 0.35
RANGE 1.00

ORDERED Y'S

0.00
0.00
0.00
0.00
0.11
0.20
0.20
0.45
0.56
0.67
0.73
0.73
1.00

****

****
* MANN-WHITNEY *
* TEST *
****

SUM OF X RANKS
141.5

STANDARD
NORMAL
DEVIATE=
-1.74

T1= 141.50

****

****
* MANN-WHITNEY *
* TEST *
* FOR *
* INDEPENDANT *
* SAMPLES *
****

2.80
0.45
1.00
2.00
0.69
0.20
0.00
0.11
0.11
0.33
5.60
0.00
0.00

N 13.00
MEAN 0.73
MEDIAN 0.00
MIDRANGE 0.50
VARIANCE 0.98
STD. DEV. 0.28
RANGE 1.00

ORDERED X'S

0.00
0.00
0.00
0.00
0.00
0.00
0.00
0.08
0.09
0.11
0.11
0.20
1.00

****

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PP No. 5F1560 6/14/78

Mann-Whitney test

Nos. Live Young/100 Implantation Sites

Control vs. 30-mg/kg nitrosoglyphosate grp.

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*  DATA ENTRY FOR    *
*  INDEPENDANT       *
*  SAMPLES           *
**********************
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LIVE YOUNG PER 100 IM PLANTATION SITES FOR EACH LITTER

X

~~LOW DOSE~~ CONTROL

1.00
0.92
0.00
1.00
0.91
0.00
1.00
0.89
0.05
0.70
1.00
1.00
0.00

N	13.00
MEAN	0.72
MEDIAN	0.91
MIDRANGE	0.50
VARIANCE	0.17
STD. DEV.	0.42
RANGE	1.00

ORDERED X'S

0.00
0.00
0.00
0.70
0.89
0.89
0.91
0.92
1.00
1.00
1.00
1.00
1.00

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LIVE YOUNG PER 100/M PLANTATTION SITES FOR EACH LITTER

0.05
0.27
0.27
0.30
0.44
1.00
0.95
0.90
0.33
1.00
1.00
1.00
0.00

N	13.00
MEAN	0.54
MEDIAN	0.80
MIDRANGE	0.50
VARIANCE	0.12
STD. DEV.	0.35
RANGE	1.00

ORDERED Y'S

30 mg/kg

0.00
0.27
0.27
0.33
0.44
0.55
0.80
0.80
0.89
1.00
1.00
1.00
1.00

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**********************
*  MANN-WHITNEY      *
*     TEST           *
**********************
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SUM OF X RANKS=
102.5

STANDARD
NORMAL
DEVIATE=
0.67

TIE 186.50

"137"

****

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	28	38	155	50
Positive control**	---	---	>10³ --	>10³	>10³	433	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	66	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