UNITED ST. ES ENVIRONMENTAL PROTECTION GENCY

DATE: September 26, 1978

SUBJECT: Glyphosate, review and evaluation of TOX data (1) Roundup Reoperator exposure and re-entry data on glyphosate and N-nitrosoglyphosate and (2) Two-part study on animal metabolism of N-nitrosoglyphosate. Ac.Nos. 233914 and 3.

FROM: Mary L. Quaife, Ph.D., TB/HED MLQ, 10/3/78 E 10/11/78

TO: Mr. Robert Taylor, Product Manager, RD

Reg. No. 524-308

Monsanto Company
St. Louis, Missouri

SUMMARY:

Following Tox data are reviewed for addition to our files on Roundup and on glyphosate pesticide petitions:

"Herbicide applicator exposure to N-Nitrosoglyphosate (NNG) during application of Roundup herbicide and field re-entry, Accession No. 233914."

Details of the review are on pp. 2-6, inclusive, of this memo. Evaluation is on pp. 7-8. Conclusions are on p. 8.

In general, we find that the study as conducted, shows no detectable exposure to NNG by operators or during re-entry. We do fault the design, for lack of graduated levels of dosage, and suggest that it would be well to pursue the question as to whether the "artifactual NNG," found on analysis of glyphosate, is real NNG. We mention the good and bad parts in execution of the study.

"Metabolism of N-nitrosophosphonomethylglycine in the laboratory rat," Accession No. 233913.

Details of this rat metabolism study on N-nitrosophosphonomethylglycine (N-nitrosoglyphosate; NNG) are given on pp. 9-10 (part 1) and pp. 10-12 (part 2). Conclusions are on p. 10, para. 2, and p. 12, bottom of p.

In general, the studies are regarded as well conceived and carried out. Both the single-dose study (Part 1) and the multiple-dose study (Part) fail to give indication for any significant - if any - conversion of NNG to glyphosate or AMP in mammalian (rat) metabolism of glyphosate.

Following report is summarized and evaluated below:

"Herbicide applicator exposure to N-nitrosoglyphosate during application of Roundup® herbicide and field re-entry," by D. D. Arras, D. D. Baird, R. G. Danhaus, J. E. Cowell, R. J. Daniels, R. Lauer, L. M. Horner, A. D. Kern, C. F. Ludeke, C. M. Lottman, R. M. Kramer, P. E. Rogers, Monsanto Agr. Res. Dept., Rept. No. MSL-0288, April 1978, Accession No. 233914.

Object of Study. To determine possible amount of N-nitrosoglyphosate (NNG) an applicator may be exposed to (1) in mixing and applying Roundup and (2) on re-entering the field between 1 and 7 days after herbicide application.

(N.B. A previous report gave results of preliminary test for application, not re-entry, exposure, comprising one each application of Roundup by spraying with boom, handgun, and backpack sprayers and one tank fill. Rev. of R. Gessart, 3/30/78.)

Method. Near Pahokee, Florida, exposure was measured for tests comprising four tank fills, three each of spraying with boom, handgun, and backpack sprayers, and three each of operator re-entry exposures at 1, 3, and 7 days. (These tests include those reported preliminarily.)

The Roundup used contained 0.14 ppm NNG by chemical analysis.

For boom applications, the spray boom was set at 6 feet above ground and pulled by open cab tractor. Tank was filled with 500 gals water containing 9 gallons of Roundup. As used, an application rate of 3.09 lbs of glyphosate acid per acre (1.03 gallons Roundup per acre) was maintained. In handgun application, the operator used similar equipment except that a handgun was connected to the spray rig and carried by operator walking behind the tractor. Tank contained 1% v/v Roundup in water. Backpack sprayer contained 1.3% v/v solution of same.

Measurements of glyphosate and NNG in air and on gloves, gauze pads and treated foliage were made, in order to sample Roundup in air breathed, deposited on exposed skin surfaces, and deposited on covered skin surfaces.

Sampling technics included collection of (1) air samples (deposition on a 4-inch-diameter glass fiber/organic binder filter pad) at a volume ca. 15-20 times the amount a person normally inhales; (2) 4- x 4-inch gauze pads placed on each of eleven locations on the operator's body - 8 exposed and 3 under clothing; and (3) white cotton gloves worn by operators.

Varying climatic conditions with respect to temperature, humidity, and wind speed and direction were encountered during both application and operation re-entry.

Control gauze and air pads and both glyphosate- and NNG-fortified ones were also used to check methodology.

Herbicide-treated plots had weeds in height, ranging from low to body-high.

For re-entry exposure, a dummy, consisting of 4-inch diameter plastic pipe, had five gauze pads each attached at strategic heights to simulate body exposure. It was attached to a car which was driven slowly through the treated plots to simulate worker "walk-through." In addition, a 4-inch-high circle of pant leg (at ankle height) was taken. An air sampler, mounted on dummy at head height, was used. Finally, samples of treated foliage from each re-entry were collected.

Two boom plots and one handgun plot were used as re-entry test sites; either operator or dummy attached to a car went through each of them (for 1.5 miles' distance at 28-44 minutes of elapsed time) on days 1, 3, and 7 after Roundup application. Weather included clear, dry and foggy, wet.

To assess photodecomposition, gauze pads fortified with both NNG and glyphosate were exposed to direct sun in the field; samples were collected every fifteen minutes.

After each exposure, pads or gloves were placed in separate bottles and stored frozen until analyzed.

For analysis of gauze and air pad samples, high pressure liquid chromatography (HPLC) was used. Following sample extraction and concentration, glyphosate was separated on a HPL chromatograph column fitted with a ninhydrin post-column reactor. The depth of color formed (which would be proportional to the amount of glyphosate present) was measured by means of an ultraviolet light (UV) detector. NNG was separated on a second HPL chromatograph fitted with a post-column "Griess" reactor which hydrolyzes it to nitrite and provides color proportional to the NNG present. (The nitrite diazotizes sulfanilamide which reacts with N-(1-naphthyl)-ethylenediamine to give a colored dye.) The depth of color was measured a UV detector to determine amount of NNG present. Reliable detection limits found are 1.0 and 0.5 micrograms/sample, respectively, for glyphosate and NNG. "Reliable detection limit" is defined as peak height 10 x that of baseline "noise."

Report states that artifactual production of NNG from glove samples occurred during the evaporation step of the aforesaid analytical procedure.* Therefore, for glove samples, glyphosate and NNG were separated on an anion exchange column prior to HPLC analysis of each, as described above.

For foliage samples, a previously described method (nature not described in this report) was used with final detection of NNG as noted above. Detection limits are said to be 0.04 ppm.

For each set of exposed field samples, laboratory checks and fortifications were done. Field checks and fortifications were analyzed, also.

* This is defined as "color medium to high levels of glyphosate was present."

Results. In general, exposure samples analyzed failed to show detectable N-Nitrosoglyphosate (NNG).

Glyphosate occurred on treated weeds, as expected, and on exposed gloves. As the object was to determine NNG exposure, we do not give detailed glyphosate findings.

Although parts of the experiment were well designed so as to determine possible human exposure to NNG during application of Roundup and during re-entry, yet we believe there are major flaws in design.

Moreover, much of the analytical data appear irregular, and the report is full of explanations as to why apparent glyphosate or NNG findings do not truly indicate the presence of glyphosate or NNG, as noted (pp. 4-5, this memo) below.

Detailed results. For check samples (i.e., those supposed to contain no glyphosate or NNG), 3/99 showed detectable quantities (0.72-9.3 microg) of NNG, but 16/99 showed detectable amounts (1.0-47.3 microg) of glyphosate. However, this would, "only tend to somewhat overstate glyphosate exposure," according to the report.

Recovery values on gauze pads fortified at 1-50 microg/pad in both field and laboratory average 73.1% for glyphosate (omitting one "outlier") and 80.5% for NNG.

Air filter pads, fortified at 1-50 microg. in both field and laboratory, show recoveries of 87.4% for glyphosate and 97.0% for NNG, if 4/26 recovery values for glyphosate, ranging from 199-530% and 3/26 for NNG, ranging from 216-226% are omitted.

Corresponding values for similarly fortified (1-50 microg/sample) laboratory and field glove and denim samples are 89.3 and 80.9%, if 10/38 of the glyphosate values (220-1,320%) for recovery are omitted. (We note, five each were at 1-microg, and five each were at 5- and 10-microg fortification levels.) The report ascribes a large proportion of these high recovery values to "possible glassware and equipment contamination." Such contamination would evidence poor "quality control" of the glyphosate analytical procedure.

Analysis of exposed gauze pads showed less than detectable (0.5-microg) amounts of NNG on 235/240 samples and 0.7-1.9 microg NNG on the remaining five. The report states, however, that these NNG values are not "real," for lack of glyphosate on four of these same samples (and only 2.1 microg glyphosate on the fifth); because,"....no NNG would be found in the absence of glyphosate."

Analysis of exposed gloves showed 12/40 samples to have ca. 1-60 microg "NNG" per glove. However, these are said to be artifactual values, not real ones. The NNG was, "form(ed) artifactually during the evaporation step when medium to high levels of glyphosate were present." Other glove samples were extracted and glyphosate and NNG separated on an anion exchange column prior to any evaporation step and analysis. Limited data are given to show that the "revised" method realistically shows no (less than 0.5 microg) NNG and no glyphosate on (each of two) glove check samples; 1.9 and 2.5 microg NNG are found on (each of two) glove samples each fortified

with 2.5 microg NNG (and 500 microg glyphosate). The original method showed 9.6 and 3.5 microg NNG/sample on each of two glove samples fortified with 500 microg glyphosate and no NNG.

No mechanism for such formation of NNG from glyphosate is described; although we note the NNG is "formed artifactually," only in the presence of glyphosate. It is not made clear, really, whether the "artifactual NNG" is supposed to be actual NNG formed during preliminary analytical procedures (e.g., evaporation of water extract of a glove) or a non-NNG substance (e.g., nitrite ions from some other source which "analyzes" like NNG? If the former is the case, one wonders whether there are not many other situations and conditions under which NNG might form readily from glyphosate - in which case persons exposed to glyphosate might also be exposed to appreciable fractions (of glyphosate) as NNG, with whatever toxic hazard that might entail. It would seem that further study of conditions under which NNG might possibly form from glyphosate may well be in order.

Air samples (filter pad = 4-in. diameter) showed no (less than 0.5 microg) detectable NNG. Glyphosate found ranges from less than 1 to 104 microg/filter pad, representing ca. 35 m^3/hr for ca. 3/4 hr. These represent spray droplets, primarily; since glyphosate is non-volatile. (Report states that a found value of 183 microg/filter pad of glyphosate is not "real;" since "tall weeds (brushed) against the air sample pad.)

Foliage, at 1, 3, and 7 days after treatment with Roundup, showed ca. 200-300 ppm glyphosate on day 1, ca. 15-35 ppm on day 3, and ca. 10 ppm on day 7. No NNG was detected by the analytical method, said to be sensitive to 0.04 ppm.

Dissipation in sunlight of NNG on gauze pads (containing before exposure 1 to 50 microg each of glyphosate and NNG) occurred, with an estimated half-life of 15-30 minutes. Glyphosate, however, is said to have been stable to sunlight.

Roundup used assayed 0.4 ppm NNG. However, contrary to the expected 0.0016 ppm in normally mixed spray tank, 2/7 samples tested assayed 0.68 and 0.44 ppm NNG! (Five samples not detectable (less than 0.02 ppm) NNG.) Report "cannot explain" these values; water used came from a different canal, which "which may have some bearing" on them.

However, it is stated (without giving glyphosate analytical results for any of the seven sprayer solutions) that ratios of NNG to glyphosate in the two samples are 1.0 and 1.2 x 10^{-4}, calculate of values of glyphosate are 3% in Roundup and 0.06% in these seven spray solutions.

"Discussion." Under this title, the report assumes (1) breathing rate = 1.8 m^3/hr by applicator; (2) ca. 1,200 cm^2 bare skin is exposed by applicator; and (3) dermal absorption by applicator is ca. 10%.

With (1) use of these assumptions; (2) use of high and low figures for glyphosate content of exposure samples; and (3) calculation of NNG based on ratio of NNG/glyphosate in either product or spray mixture, the report presents a range of theoretical "exposure" values for NNG by operator by inhalation and dermal absorption. For total operator exposure, dermal and inhalation values are combined. From combined calculated exposure levels, an average exposure (to NNG) value is calculated, using a "weighted" average NNG/glyphosate ratio.

Results of these calculations are: Inhalation exposure of NNG is 4×10^{-8} to 8×10^{-4} microg/hr. Dermal exposure of NNG is ca. 4×10^{-3} to 2×10^{-1} microg/hr. Combined values range from ca. 4×10^{-6} to 2×10^{-1} microg/hr. The average combined exposure is said to be ca. 10^{-2} microg NNG/hr.

Tank filling is said to add no significant amount of NNG exposure exposure; average calculated exposure of boom and handgun application is 10^{-2} microg NNG/hr.

Re-entry exposure (said to be due only to absorption from the skin picking up residues on weeds) is calculated to average ca. 5×10^{-3} microg/hr.

All above values are considered as maximal; since the NNG/glyphosate ratio used was that determined on the spray solution with much higher than expected NNG content found by analysis for NNG.

However, the report reiterates: "N-nitrosoglyphosate (NNG) was not detected on any of the sampling media on operators re-entering treated fields as early as one day after treatment." And, "Real levels of NNG were not detected on any of the sampling media taken from operators using spray solutions, which contained either non-detectable or detectable levels of NNG."

Conclusion. According to this report, no NNG, detectable by chemical analysis (i.e., less than 0.5 microgram/sample) was detected in exposure samples. Therefore, by use of a "weighted average ratio" of NNG/glyphosate, (in Roundup and in diluted spray samples), report authors have calculated that average combined exposure to N-nitrosoglyphosate by dermal and inhalation routes during boom and handgun applications of Roundup is 10^{-2} micrograms per hour (tank filling, adding no significant exposure). Re-entry exposure is calculated to average 5×10^{-3} micrograms per hour.

EVALUATION:

In general, we agree with the report's conclusion that the study as conducted fails to show detectable exposure to NNG, either of operators or during re-entry.

We did say (p. 4, this memo), we think that there are major flaws in the experimental design.

The main flaw we find is the use of only one treatment-level of Roundup. We wonder why such a study did not use graded levels of treatment. In this way a dose-response curve of residues of NNG in the exposure-samples to levels of treatment could be constructed and a much more accurate picture of "true exposure" to NNG should emerge. (Such ~~multiple~~ multiple treatment- or dose-levels are commonly used in determining residue levels on treated r.a.c.'s or in meat, milk or eggs for tolerance-setting purposes.)

Secondly, if the amount of NNG in Roundup is as high as 0.4 ppm, why not use this product to obtain maximum exposure at recommended dose? (The Roundup actually used had 0.14 ppm NNG.)

OK to decline B

Even better, might be the use of pure NNG at graded levels, the lowest one corresponding to the amount in Roundup when used as directed on its label.

Use of the single treatment-level means that nearly all assays for NNG had to be conducted at or below the sensitivity (limits of detection) of the assay method. In such case, somewhat small absolute errors in assay results for NNG become large in relative terms. E.g., "recovery" of 2 micrograms, when only one microgram was added, becomes a relatively high "200%.")

In addition to design flaw, we fault the study for failure to ensure that all seven diluted spray solutions used assayed for NNG according to theoretical content.(0.0016 ppm). Two of the seven assayed at 0.4 and 0.7 ppm NNG; while the other five showed less than 0.02 ppm (method sensitivity).

OK to decline B

We fault the study for not pursuing further the question of of artifactual production of NNG during analysis of certain samples. This is said to have taken place in presence of moderate or high amounts of glyphosate on exposed glove samples. Extremely limited data (total of six assays) are presented to demonstrate that a revised method (which first separates NNG from glyphosate before detection of the former) does not give rise to such artifactual NNG.

We are not informed, nor can we judge accurately from meager experimental data provided, whether, in fact, the so-call artifactual NNG is really NNG formed during the analysis. If it <u>is</u> NNG, one wonders whether there may not be other situations wherein NNG might form and exert its toxic effects, if any, upon exposed persons. We believe the problem should be pursued further to show whether or not the "artifactual NNG" <u>is</u> NNG.

Also, quality control in chemical analysis was evidently poor.

For glyphosate analysis, 25% of fortified glove and denim samples showed high recovery values (200% to 1,200%) due to "possible glassware and equipment contamination," according to the report. Also, 16/99 check samples (which are supposed to contain no glyphosate or NNG) showed detectable amounts (1-47 micrograms) of glyphosate.

Anomalous values for NNG found are far fewer. However, we point out that the report explains away the finding of detectable levels of NNG on 5/240 gauze pads analyzed as not "real," because NNG "would not be found in the absence of glyphosate."

CONCLUSIONS:

1. The study as carried out fails to show detectable exposure to NNG, either by operators or during re-entry at 1 to 7 days after treatment, by either of the two possible routes, inhalation or absorption through the skin.

2. The study was well set-up and carried out with respect to placement and collection of exposure samples. It covered three types of application, tank-filling operations, and exposure during re-entry. A variety of weather conditions and a variety of foliage-heights were encountered.

3. However, we do believe a major flaw in design of the study was failure to use graded levels of dosage with Roundup, as is commonly done in residue studies for tolerances, in order to give a dose-response curve and, thereby, provide a true picture of NNG exposure by operating personnel. Possibly, pure NNG should be used in such a study.

4. We question as to whether the problem of "artifactual NNG" which was shown to be formed on certain exposure samples in presence of medium or large amounts of glyphosate, should be pursued further to determine whether, in fact, under certain conditions NNG is formed from glyphosate. If true, one might want to know whether there are any implications for hazard evaluation of proposed uses of glyphosate (Roundup).

5. Poor quality control in the analysis of glyphosate is in evidence in this study. Both check and fortified samples showed a large proportion of false high values in two series.

Review of TOX data.

Metabolism of N-nitrosophosphonomethylglycine in the laboratory rat, by M.L. Sutherland, Monsanto Agr. Res. Dept., 5/16/78, Accesion No. 233913.

Part 1.

Method. After 12-hr fast, groups of 3M and 3F young adult rats each received by stomach tube a single dose of 1 ml of solution containing 0.986 mg N-nitroso-glyphosate (NNG) C13/C14 (ca. 12 x 10^6 DPM/ml = 5.43 microcuries) dissolved in water or water alone.

Rats were each placed in metabolism cages, and they received food and water ad lib.

All rats were transferred once a day to holding cages; these and metabolism cages were rinsed with deionized water and respective excretion products composited with main samples.

All rats were killed (by CO2) 5 days after dosage, frozen with dry ice, and stored frozen.

C14-contents of urine samples and "cage rinses" were determined by liquid scintillation counting (LSC). Filtrates of acetone slurries of feces samples were counted likewise; while the resultant dry feces powders were combusted and the C14O2 collected for LSC.

Sufficient first-day urine samples of treated rats were mixed and sequentially chromatographed on [H+] cation exchange resin, [HCO3-] resin, and again [H+] resin to isolate nitrosoglyphosate. This was done either with or without adjustment of the solution to pH 8-9 with NH4OH prior to concentration.

Finally, this isolated material was examined by nuclear magnetic resonance (NMR) spectroscopy with Fourier transform, with use of C13-ethanol as internal reference. Also, a derivative of the isolated material was made by reaction with diazomethane, and the derivative examined by gas-liquid chromatography/mass spectroscopy (GLC/MS).

Results. No data (C13/C14) on control rats are given; either they were not examined or, presumably, gave negative findings.

All rats gained weight. Two control females could not be gavaged. One test female rejected one-third of test dose, and results found are not included in the summarized values for C14 excretion:

AVERAGE MALE C14 EXCRETION

Males:	Total Urine	88.7%	Sum 91.5%
	Total Feces	2.8%	

AVERAGE FEMALE C14 EXCRETION

Females:	Total Urine	80.8%	
	Total Feces	10.7%	Sum 91.5%

Nearly all (greater than 98%) of total C14 was excreted in the first 24 hrs.

Review of TOX data (contd.)

Part 1. (contd.)

Chromatographic and C13-NMR data on urine combine to show that, during the chromatographic purification and evaporation without NH_4OH treatment, the NNG was converted to glyphosate. Same type examination showed that with NH_4OH treatment, the urinary NNG was not converted to glyphosate. In latter case, GLC/MS examination of the isolated material confirmed that it was indeed unchanged N-nitrosoglyphosate.

Conclusion. The excretion of N-nitrosoglyphosate fed to rats in a (1-mg) single oral dose is - in urine and feces - in the form of unchanged N-nitrosoglyphosate. More than 90% of dose fed (as C14) is so accounted for.

Part 2.

Method. Groups of 2M and 2F young adult rats each received by stomach tube a dose of 5.93 mg (30 mg/kg body weight) of N-nitroso-glyphosate C13/C14 (ca. 6.7×10^7 DPM) in ca. 2 ml (?) water or water alone, daily, for 5 days.

Rats were each placed in separate metabolism cages and, presumably, received food and water ad lib.

All rats were transferred once a day to holding cages; these and metabolism cages were rinsed with deionized water (former only if necessary) and respective excretion products composited with main samples for the day.

All rats were killed by CO_2, 24 hrs after the last dose, frozen, and stored frozen.

Urine samples, cage rinses, and fecal samples were processed for determination of C14-content as described in Part 1 (preceding page).

However, since solids began to precipitate out of urine from control and treated rats during the 24-hr collection period in the metabolism cage, special attention was given to determine whether such urine solids from treated animals contained any C14. Three urine samples from rat (F-606) which had largest volume of solids were passed through Gelman membrane filter and the membrane plus solids air-dried and analyzed by combustion analysis (with resultant $C^{14}O_2$ being counted by LSC).

Treated animals were thawed and dissected into following: Blood, fat, gut contents, heart, brain, spleen, reproductive organs, kidney, muscle, gut wall, lung, liver, and remaining carcass, including skin; latter was ground until homogeneous. All other tissue samples and organs were lyophilized. Aliquots of each tissue, organ, or fraction were weighed onto planchets for measurement of C14 content in a Searle Combustion Apparatus. Latter burns sample to $C^{14}O_2$, which is converted to carbamate in a basic trapping solution; carbamate (from each sample) is flushed into separate vial for counting by LSC.

Review of TOX data (contd.)

Part 2 (contd.)

An aliquot of each daily urine sample from each treated rat was chromatographed on anion exchange resin by a procedure that will separate (and quantitate) glyphosate, nitrosoglyphosate, and aminomethyl phosphonic acid. Those fractions that corresponded to NNG were composited from all 20 analyses. The composite was further purified on a cation exchange resin, adjusted to pH 8 with NH_4OH, concentrated to dryness, and then subjected to C13 NMR for confirmation of identity as nitrosoglyphosate.

Acetone feces powders (cf. "Method," under Part 1) from each of the four treated rats were extracted with deionized water; the extract purified on Gelman membrane filter (more than 90% C14 of feces was contained in the purified extract); and the extract chromatographed on anion exchange resin which separates NNG from two related compounds, as noted in preceding paragraph). A composite of NNG fractions from each rat fecal sample was further purified by chromatography on cation exchange resin, adjusted to pH 8, evaporated to dryness, and subjected to C13 NMR - the latter to confirm its identity as nitrosoglyphosate.

Any given organ or tissue from all four rats was composited (after individual counting for C14 content). Dry powders from each composite were prepared from a $CHCl_3$ slurry (made by grinding the composite in the presence of $CHCl_3$) by centrifuging the slurry and letting the centrifuged solid air-dry. The powder was extracted with deionized water; the extract purified on Gelman membrane filter; and the extract chromatographed on anion exchange resin, which separates glyphosate, NNG, and aminomethylphosphonic acid.

Composite, presumably NNG, fractions from urine and feces, prepared as described on the first two paragraphs on this page, were examined by means of C13-NMR (nuclear magnetic resonance) spectroscopy, the instrument being equipped with Fourier transform. In each case, an internal standard of C13-ethanol was included for reference. Purpose of this examination was to confirm, if possible, the identity of the purified material as N-nitrosoglyphosate.

Results. All rats gained weight during dosage; however test rats gained, on the average, less than one-half as much as corresponding controls.

Except for an NMR spectrum (C13) of control rat urine composite, no C13/C14 data on control animals are given in the report.

C14 excretion data are as follows:

	AVERAGE MALE C14 EXCRETION		
Males:	Total Urine	60.4%	Sum 94.0%
	Total Feces	33.6%	
	AVERAGE FEMALE C14 EXCRETION		
Females:	Total Urine	63.0%	
	Total Feces	29.3%	Sum 92.3%

Review of TOX data (contd.)

Part 2 (contd.)

Daily combined urinary and fecal excretion of C14 was fairly constant from day 1 for these rats; therefore, apparently, an equilibrium period was not required for saturation of tissues or organs with C14.

In contrast to results of Part 1 of the study (above), this multiple dose schedule, using 5 x the amount given in Part 1/each day, resulted in excretion of ca. 61% of C14 in the urine (and ca. 31% in feces). This may mean that the higher dose (30 mg/kg BW) saturates the capacity of the rat stomach.

C14-content of organs and tissues was low. The carcass had 0.2 to 0.3% of total dose. Liver was next with 0.02%. All other organs (and gut contents) contained less than 0.01% each.

If it is assumed that all C14 represents unchanged NNG, then average content of various fractions, in ppm, is, as follows: Muscle, 0.02; fat, 0.04; heart, 0.04; brains, 0.09; blood, 0.09; reproductive organs, 0.10; gut wall, 0.14; kidney, 0.26; carcass, 0.32; spleen, 0.36; liver, 0.56; lung, 0.88; and gut contents, 3.40.

Chromatographic evaluation of C14 in each of the 20 urine samples showed more than 99.9% to be NNG in all but three of them. These three were the ones in which solids were separated and tested for C14-content (negatively so); in these three, detectable amounts of glyphosate and of aminomethylphosphonic acid were found - AMP = 0.6 to 1.7 and glyphosate = 0.0 to 0.5%.

The authors ascribe these apparent findings of AMP and glyphosate in the three urine samples to photolysis and hydrolysis of some NNG C13/C14 during filtration of the solids from them. We believe this is a reasonable surmise.

Water extracts of composite fecal samples from each treated rat were likewise evaluated by chromatography. There were detectable amounts of AMP C13/C14 (0.3 to 0.5%) and in one case 0.08% of glyphosate. Authors again ascribe the presence of these to photolysis or hydrolysis of NNG.

Similar evaluation of composited organs and tissues revealed no indication of AMP or glyphosate present in any of the chromatograms.

Finally, C13-NMR spectroscopy of chromatographic eluates corresponding to NNG - in the case of both urine and feces - revealed spectra which are consistent with that of NNG.

Conclusion: Results of this multiple-dose study on NNG given to rats fail to give indication for any significant - if any - conversion of NNG to glyphosate or AMP in any body organs or tissues.