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Atrazine / Review # 58 / 11-6-86 / 24 pages

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY

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MEMORANDUM

SUBJECT: Atrazine - Review of Metabolism Studies in the Rat (EPA Registration No. 100-529) in Support of Reregistration of Atrazine

TOX. Chem. No. 63

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Registrant: CIBA-GEIGY Corporation

Accession Number: 255815

Toxicology Branch has reviewed two studies concerning metabolism of atrazine (2-chloro-ethylamino-6-isopropylamino-S-triazine) in rats, entitled:

- Dermal Absorption of ^{14}C-Atrazine by Rats
- Excretion Rate of ^{14}C-Atrazine from Dermally Dosed Rats

The following comments pertain to the results of each study:

A. Dermal Absorption of ^{14}C-Atrazine by Rats: Male and female Harlan Sprague-Dawley rats were dosed dermally with ^{14}C-atrazine at either 0.25 or 2.5 mg/kg. For the "Balance Phase" of the study CO_2, urine, and feces were collected at 24, 48, and 72 hours after treatment and analyzed for radioactivity. The animals were sacrificed 72 hours posttreatment. For the "Kinetic Phase" of the study, four treated animals of each sex were sacrificed at 2, 4, 8, 24, or 48 hours after treatment, major organs and tissues dissected out and analyzed for radioactivity. Due to the low solubility of ^{14}C-atrazine in ethanol, the authors considered the results obtained from the high dose level (2.5 mg/kg) unreliable, and thus only data from the low dose (0.25 mg/kg) were considered. The results of this study indicate that ^{14}C-atrazine is slowly absorbed from the skin (approximately 70 percent of the applied dose in 72 hours) of male and female animals resulting in a half-life absorption of approximately 41 hours for both sexes. The radioactivity was distributed mainly into muscle and liver in both sexes at all time points examined. Most of the atrazine-derived radioactivity absorbed from the skin was excreted in the urine (approximately 35 percent) and to a lesser extent in the feces (approximately 11 percent) in both sexes within 72 hours after application. Analysis of urine (obtained from animals treated with the low [0.25 mg/kg] or high dose [2.5 mg/kg] of ^{14}C-atrazine) indicated that approximately 82 percent of the radioactivity was in the form of ^{14}C-atrazine or atrazine metabolites which upon hydrolysis are converted to a common moiety, ^{14}C-cyanuric acid.

This study was classified as Core-Supplementary based on the following deficiencies (see also attached DER):

1. The radiochemical purity of ^{14}C-atrazine was not supplied.

2. The authors should supply the Agency with more information regarding atrazine metabolites in urine and feces and provide data as to how methyl cyanuric acid was identified and quantified.

The study can be upgraded to Core-Guideline classification when the issues cited above are resolved.

B. Excretion Rate of ^{14}C-Atrazine From Dermally Dosed Rats: Male and female Harlan Sprague-Dawley rats were dosed dermally with ^{14}C-atrazine (two rats/sex/dose) at dose levels of 0.025, 0.25, 2.5, or 5 mg/kg in a total volume of 15-20 uL. Urine and feces were collected from all animals at 24-hour intervals for 144 hours when the animals were sacrificed. Analysis of urine indicated that the percent of atrazine-derived radioactivity excreted in the urine in 144 hours was increasingly higher with higher dose levels tested ranging from 37 percent (lowest dose) to 59 percent (highest dose). Most of the radioactivity for all dose levels was excreted in the urine within 48 hours after application. Atrazine-derived radioactivity excreted in the feces in 144 hours was approximately 15 percent for the lowest dose tested (0.025 mg/kg) and between 20 and 21 percent for the rest of the dose levels. Most of the radioactivity in feces was excreted within 72 hours after treatment. The cumulative excretion of atrazine-derived radioactivity in urine and feces in 144 hours was 52 percent with the lowest dose and 70 to 80 percent with the rest of the dose levels.

This study was classified as Core-Supplementary based on the following deficiencies:

1. The radiochemical purity of atrazine was not reported.

2. Although several organs/tissues were collected from all treated animals, these organs/tissues (or carcasses) were not analyzed for radioactivity content for purposes of calculating recovery accurately.

3. The authors failed to report the nature of the radioactivity (parent compound/metabolites) excreted in urine and feces.

The study can be upgraded to Core-Guideline classification when the above deficiencies are resolved.

Subject: Excretion Rate of ^{14}C-Atrazine from Dermally Dosed Rats

Test Material: ^{14}C-Atrazine [2-chloro-4-(ethylamino)6-(isopropylamino)s-triazine].

Accession Number: 255815

Sponsor: CIBA-GEIGY Corporation, Greensboro, NC.

Testing Facility: CIBA-GEIGY Corporation, Greensboro, NC.

Report Number: ABR-83081

Testing Period: Not specified

Report Submitted to Sponsor: October 1983.

Materials and Methods:

Eight female Harlan Sprague-Dawley albino rats (obtained from P.O. Box 4220, Madison, Wisconsin) weighing approximately 200 g each were used for this study. The animals were prepared for dermal application by shaving the dorsal hair with electric clippers, leaving the stratum corneum intact. The animals were divided into 4 groups (2 rats each) and dermally treated with ^{14}C-atrazine at dose levels of 0.025, 0.25, 2.5 or 5 mg/kg. The appropriate dose of ^{14}C-atrazine (specific activity 17.2 uCi/mg) dissolved in tetrahydrofuran was applied to 1.5 cm^2 area of the shaved skin at a total volume of 15-20 uL. (Note: the radiochemical purity of ^{14}C-atrazine was not reported by the authors.) To prevent scratching of the treated area the rear legs of the rats were shackled with a chain. After treatment the rats were individually housed in stainless steel metabolism cages (Acme, Division of Noeltge, Inc., Cincinnati, Ohio) allowing for separate collection of urine and feces. Food (name not specified) and water were available to all animals ad libitum.

Urine and feces were collected from each animal at 24-hour intervals for 144 hours. The rats were sacrificed 144 hours after treatment and the lungs, heart, spleen, kidneys, and liver were immediately dissected from each animal and, along with carcasses, were stored for future use. The exposed skin was washed in 100 mL of tetrahydrofuran for 24 hours and then placed in 5 mL of Beckman 450 solubilizer and the radioactivity counted as described below.

Radioassay Procedures:

Cage wash, skin wash, and urine were radioassayed directly using Scintiverse as the scintillation fluid while feces were first combusted and then radioassayed using oxifluor-CO_2 as the scintillation fluid. All counting of radioactivity was carried out in a Mark III Model 6881 or Tracor 6895 liquid scintillation counter and efficiencies were determined using external standards. ^{14}C-Mannitol was used in determining combustion efficiencies.

Results:

The present study has investigated the excretion of ^{14}C-atrazine at different time points following a single dermal application to rats at four different dose levels. Table I (abstracted from the original report) shows the percent of the dose excreted in urine and feces at 24, 48, 72, 96, 120, or 144 hours after exposure. The total atrazine-derived radioactivity excreted in the urine varied with the dose being 36.9% with the lowest dose tested (0.025 mg/kg) and 58.8% with the highest dose tested (5.0 mg/kg). Most of the radioactivity for all dose levels was excreted within 48 hours after exposure.

The total atrazine-derived radioactivity excreted in the feces (expressed as a percent of the dose) was 14.9% for the low dose (0.025 mg/kg) and between 20% and 21% for the rest of the dose levels (Table I). As was the case with excretion in the urine, most of the radioactivity was excreted in feces within 48 hours after application (with the exception of the 0.25 mg/kg dose level where high excretion was observed in feces at the 72- and 96-hour time points). The cumulative excretion of atrazine-derived radioactivity in urine and feces at 144 hours after application appeared to be higher with increasing dose, varying between 52 and 80 percent from the lowest to the highest dose, respectively.

The quantities of atrazine-derived radioactivity, expressed as ug of atrazine, excreted in the urine and feces are shown in Table II (abstracted from the original report). These data indicate that the quantities of atrazine excreted in the urine and/or feces are directly proportional to the dose. The amount of atrazine-derived radioactivity (as percent of dose) remaining on the application site (surface of exposed skin) was reported by the authors to be inversely proportional to the applied dose ranging from approximately 10% with the lowest dose (0.025 mg/kg) to 1% with the highest dose tested (5.0 mg/kg) (Table III). The percent of the dose remaining in the skin (dissolved) was very low accounting for less than 1% of the applied dose for all dose levels, although, as seen in Table III, the higher the dose the lower the percent retained in the skin.

Discussion:

The authors failed to report the radiochemical purity of atrazine used in this study. It also appears from the authors' report that nonlabeled atrazine was not used for this study. Instead, for obtaining the desired dose level, the authors used higher amounts of radioactivity (specific activity for ^{14}C-atrazine was 17.2 uCi/mg). Thus for the lowest dose (0.025 mg/kg) a total of 291,167 DPM's were applied to each rat while for the highest dose (5.0 mg/kg) a total of 37,538,833 DPM's were applied to each rat (see appendix). This tremendous difference in the amount of radioactivity (approximately 129-fold difference between lowest and highest dose tested) introduces additional variability between dose groups and is not recommended.

The present study contains additional deficiencies as follows:

1. Although the authors reported that several organs were dissected from each animal, these organs were not analyzed for radioactivity content. The radioactivity retained in tissues/organs is essential in estimating total recovery of the applied dose.

2. The authors did not indicate whether the absorbed dose of atrazine is excreted in the urine and feces in the form of metabolites or as parent compound or both. This information is essential in establishing metabolic pathways for atrazine in the rat and determining whether these pathways are affected by dose (become saturated at higher doses) or not.

   Data presented here (Tables I, II, and III) indicate that absorption of atrazine from the skin and excretion of atrazine and/or metabolites (in urine and feces) are directly proportional to the dose. Both, absorption and excretion processes, do not appear to be saturable at the dose range studied. The facilitated excretion observed with the higher dose levels suggests that retention of atrazine in the body is lowered. However, since metabolism of atrazine in different tissues was not investigated, it is not clear whether facilitated excretion results from induction of enzymes responsible for atrazine metabolism or by affecting some other physiological mechanism.

Conclusions:

Atrazine, applied to the intact skin of the female rat at dose levels of 0.025, 0.25, 2.5, or 5.0 mg/kg, is readily absorbed and within 48 hours most of the absorbed dose is excreted mainly in is the urine and to a lesser extent in the feces. Absorption from the skin and excretion in urine and feces appear to be

directly proportional to the dose tested.

Classification:

The present study is classified as Core-Supplementary based on deficiencies listed above.

TABLE I: PERCENT OF DOSE RECOVERED IN RAT EXCREMENT HOURS AFTER TREATMENT[a]

		% of Dose Hours After Treatment						
	Dosage Level	24	48	72	96	120	144	Total
Urine								
	.025	16.50[a]	10.7	4.15	2.52	1.42	1.61	36.9
	.25	12.79	10.35	9.93	8.19	4.87	4.17	50.3
	2.50	21.63	15.9	6.78	5.14	3.03	3.22	55.7
	5.0	31.49	13.38	6.55	3.72	2.08	1.84	58.8
Feces								
	.025	4.26	5.38	2.44	1.21	.71	.9	14.9
	.25	2.06	5.72	4.03	3.65	2.32	1.87	19.65
	2.5	2.47	9.69	3.36	2.39	1.42	1.23	20.56
	5.0	8.09	5.87	1.81	2.84	1.24	.75	20.6
Total Excrement								
	.025	20.76	16.08	6.59	3.73	2.13	2.51	51.8
	.25	14.85	16.07	13.96	11.84	7.19	6.04	69.95
	2.5	24.10	25.59	10.14	7.53	4.45	4.45	71.81
	5.0	39.58	19.25	8.36	6.56	3.32	2.59	79.66

[a]Average duplicate from one rat only

TABLE II: MICROGRAMS OF ^{14}C-ATRAZINE RECOVERED IN RAT EXCREMENT VS HOURS AFTER TREATMENT[a,b]

	Dosage			µg/Daily Excretion						
	mg/kg	mg	mg/cm²	24	48	72	96	120	144	Total
Urine										
	.038	.007	.004	1.26[b]	.82	.32	.19	.11	.12	2.82
	.25	.05	.033	6.37	5.16	5.07	4.07	2.42	2.07	25.16
	2.43	.48	.32	105.58	77.64	66.16	25.12	29.52	15.73	319.75
	4.9	.98	.65	309.5	131.47	64.43	36.54	20.55	18.16	580.65
Feces										
	.038	.007	.004	.33	.41	.25	.09	.07	.07	1.22
	.24	.05	.033	1.02	2.81	2.0	1.81	1.15	.93	9.72
	2.43	.48	.32	12.05	47.3	16.4	11.67	6.93	11.93	106.28
	4.9	.98	.65	79.48	57.68	17.77	27.88	12.16	7.36	202.33
Total µgs										
	.038	.007	.004	1.59	1.23	.57	.28	.18	.19	4.04
	.24	.05	.033	7.39	7.97	7.07	5.88	3.57	3.0	34.88
	2.43	.48	.32	117.63	124.94	82.56	36.79	36.45	27.66	426.03
	4.90	.98	.65	388.98	189.15	82.2	64.42	32.71	25.52	782.98

[a]Individualized data located in Appendix

[b]Average duplicate from one rat only

TABLE III: PERCENT OF DOSE FOUND IN SKIN WASHES AND DISSOLVED SKIN ONE HUNDRED FORTY-FOUR HOURS AFTER TREATMENT[a,b]

	.025 mg/kg	.25 mg/kg	2.5 mg/kg	5.0 mg/kg
Skin wash[a)]	<*9.82	4.76	3.9	.92
Skin dissolved[b)]	.72	.56	.16	.11

<*Below the level of quantitation

a)Dose left on surface
b)Dose retained in skin

APPENDIX: INDIVIDUALIZED DATA OF EIGHT FEMALES DERMALLY DOSED WITH ^{14}C-ATRAZINE

Rat #	Weight	DPMs Applied	mg/kg	Average mg/kg	mg/cm^2	Average mg/cm^2
R1037	195.700g	291,167	.038	.038	.025	.025
R1038	198.325g	291,167	.038		.025	
R1039	192.661g	1,899,667	.258	.25	.172	.167
R1040	205.668g	1,899,667	.242		.161	
R1041	202.310g	18,639,500	2.41	2.43	1.60	1.62
R1042	199.505g	18,639,500	2.45		1.63	
R1043	204.928g	37,538,833	4.80	4.90	3.2	3.27
R1044	197.211g	37,538,833	4.99		3.32	

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Subject: Dermal Absorption of ^{14}C-Atrazine by Rats

Test Material: Atrazine (2-chloro-4-ethylamino-6-isopropylamino-5-triazine)

Accession Number: 255815

Sponsor: Ciba-Geigy Corporation, Greensboro, NC

Testing Facility: Ciba-Geigy Corporation, Greensboro, NC

Report Number: ABR-83005

Testing Period: Not specified

Report Submitted to Sponsor: May 1983

Materials and Methods

Male and female Harlan Sprague-Dawley albino rats (obtained from P.O. Box 4220, Madison, WI) weighing approximately 200 g were used for this study. The animals were prepared for dermal application by shaving the dorsal hair with electric clippers without injuring the stratum corneum. Each rat was then anesthetized with ether and an area of 1.5 cm^2 was marked. ^{14}C-Atrazine (specific activity of 17.2 uCi/mg) was dissolved in ethanol so that between 15 and 20 uL of solution corresponded to the dose levels of 0.25 or 2.5 mg/kg body weight. The ^{14}C-atrazine was applied to the skin area and the radioactivity was equivalent to 2×10^6 dpm or 2×10^7 dpm per rat for the low- or high-dose levels, respectively. To prevent scratching of the treated area the rear legs of the treated rats were shackled with a stainless steel chain. After treatment, rats were individually housed in cages with food (name of diet not specified) and water available ad libitum.

The present study consisted of two phases:

A. Balance Phase: For this part of the study treated rats were placed individually in modified Roth metabolism cages (Standford Glassblowing Laboratories, Inc., Palo Alto, CA) with a dry ice acetone trap for the collection of volatiles and a 400 mL 2N NaOH trap for collecting CO_2. CO_2 was sampled at 24 hours (from all animals) and at 24, 48, and 72 hours from one male and one female of each group. A preliminary study (72-hour high dose) indicated that sampling of volatiles was not necessary during this phase of the study. Urine and feces were also collected at 24, 48, and 72 hours posttreatment. The animals were sacrificed 72 hours after treatment.

B. Kinetic Phase: For this part of the study, treated animals were individually housed in stainless steel metabolism cages (ACME, Division of Noeltge, Inc., Cincinnati, OH) and sacrificed 2, 4, 8, 24, or 48 hours after treatment. Four male and four female rats were dosed at the high- and low-dose levels for each time point.

The following tissues were taken from all treated animals of the balance or kinetic phase of the study and radioassayed as described below:

treated skin area	plasma	spleen
blood cells	brain	heart
muscle	liver	small intestine
lung	stomach	large intestine
kidney	gonads	

In order to determine what quantity of the applied dose remained unabsorbed and how much was absorbed into the skin, the treated skin area was washed in 100 mL of acetone (overnight) and then placed in 5 mL of Beckman 450 solubilizer. In an earlier preliminary study (reported as "Zero Time Samples") skin samples were taken from male and female animals, dosed with the high- or low- dose level of atrazine, placed in 100 mL of acetone overnight and then placed in 5 mL of Beckman-BTS-450 solubilizer. Aliquots of the acetone rinse and the solubilizer were radioassayed.

Radioassay Procedures: Cage wash, skin wash, solubilized skin, acetone rinse, urine, and volatiles were radioassayed directly. Expired CO_2 was radioassayed as described by Roger J.G., AG-250 (Radioassay of $^{14}CO_2$ by acid neutralization and subsequent counting by liquid scintillation). Carcasses and all other samples (tissues, feces, etc.) were homogenized with liquid nitrogen and dry ice using a Wiley Mill (for carcasses) or mortar and pestle (all other samples). Aliquots of homogenates were combusted using a Harvey Oxidizer. Combustion efficiencies were determined using ^{14}C-mannitol. All counting of radioactivity was carried out in a Mark III model 6881 liquid scintillation counter with efficiencies determined by external standardization.

To calculate percent of total ^{14}C for organs, the weights of the whole organs were used. For nonorgans, the values used were: muscle, 45.4% ; fat, 10.6% ; blood volume, 6.39% ; blood plasma, 4.04% ; and red blood cells, 2.35% of the total weight of each rat treated.

Analysis of Metabolites in Whole Urine: Urine from the 72-hour group, collected at 24, 48, and 72 hours after treatment, was analyzed for total triazine residues. Triazine residues were determined by converting them to cyanuric acid by digesting them with concentrated and dilute nitric acid (two-step oxidative hydrolysis).

Control urine was also analyzed after spiking it with the appropriate ^{14}C standard. The hydrolyzed samples were cleared from interfering salts and then analyzed for metabolites with thin layer chromatography (TLC) using toluene:acetic acid:water (10:10:1) as the developing solvent system. Radioactivity was analysed using a Berthold spark chamber and Kodak NS-2T no screen X-ray film. For quantitation, the radioactive peaks were scraped from the TLC plates (silica gel) and the radioactivity counted using a scintillation counter. Calculation of metabolic data was based solely on radioactive counting.

Results

In a preevaluation study where a female rat was dermally exposed to the high dose (2.5 mg/kg) ^{14}C-atrazine, approximately 106 percent of the applied dose was recovered as shown in Table 1. Volatiles and CO_2 accounted for less than 0.5 percent of the total dose while radioactivity recovered in the urine and feces accounted for 57 percent of the dose.

The authors reported that due to the low solubility of ^{14}C-atrazine in ethanol, the results obtained from the high dose tested (2.5 mg/kg) were considered unreliable and thus not included here in establishing the dermal absorption and pharmacokinetics of ^{14}C-atrazine in rats (although data were reported).

The balance phase of the study indicated that when rats were dermally exposed to ^{14}C-atrazine (0.5 mg/kg low dose) the recovery of the applied dose was approximately 100 percent in males and 103 percent in females, 72 hours after dosing (Table 2). Most of the atrazine-derived radioactivity absorbed from the skin was excreted in the urine and accounted for approximately 35 percent of the applied dose in both sexes. Lower quantities (11 percent of the dose) were excreted in the feces and approximately 7 percent of the dose was retained in the tissues (including blood). Animal carcasses contained up to 13 percent of the applied dose while radioactivity in cage wash accounted for 6 percent of the dose (Table 2). Unabsorbed radioactivity recovered from the application site (skin wash) accounted for 28 percent of the total dose applied.

The atrazine-derived radioactivity excreted in the urine and feces at the 24-, 48-, and 72-hour time points (balance phase) is shown in Table 3. Approximately equivalent quantities are excreted

by male and female rats at each time point. Within 24 hours after treatment approximately 14 percent of the dose is excreted in the urine and 3 percent in the feces. Between 24 and 48 hours, approximately 12 percent was excreted in the urine and 4 percent in the feces. From 48 to 72 hours lower quantities were excreted in the urine (9 percent) while excretion in feces was still at approximately 4 percent.

Results obtained from the kinetic phase of the study are presented in Table 4. The amount of atrazine-derived radioactivity remaining at the application site (skin wash), and thus unabsorbed, is ralatively high (over 80 percent) at the initial time points (2, 4, and 8 hours) in both sexes, and approximately 68 percent, 42 percent, and 29 percent of the 24-, 48-, and 72-hour time points, respectively (Table 4). The content of tissues in atrazine-derived radioactivity varied with the tissue and the time point examined. At the 2-hour time point none of the tissues contained apreciable quantities of radioactiity (mostly below quantitation levels). At the 4-hour time point approximately, 2.7 percent of the dose appears in the muscle of female rats and 0.3 percent in the liver. Muscle and liver in both sexes contained the highest radioactivity at the 8-hour time point (2.1 and 0.5 percent, respectively). Higher tissue content (muscle, liver, blood, large intestine) in radioactivity was observed at the 24-hour time point, declining slightly in all tissues at the 48-hour time point in both sexes.

From the above results the authors have calculated the half-lives for absorption of radioactivity from the skin. In male rats the half-life was found to be 38.88 hours (with a correlation coefficient of 0.995) and in females the half-life of absorption was found to be 42.97 hours (with a correlation coefficient of 0.981). Thus, the half-life of absorption for male and female rats combined was 40.9 hours.

Urine from male and female animals from the 72-hour group was analyzed (as described in Methods) for total triazine residues. The urine analyzed came from the 24- and 72-hour time point of the high-dose group males and females and 24-hour female urine from the low-dose group (Note: The authors reported that they considered the data obtained from the high-dose group, 2.5 mg/kg, to be unreliable and no conclusions should be based on these data; however, most of the metabolism results were obtained using urine from the high dose group.) The results, according to the authors, indicate that approximately 82 percent of the radioactivity in the urine is in the form of either ^{14}C-atrazine or atrazine metabolites which upon hydrolysis give ^{14}C-cyanuric acid. Only 5 percent of the radioactivity was present in the form of methyl cyanuric acid.

Discussion

The present study has investigated the dermal absorption of ^{14}C-atrazine in male and female rats after application of either 0.25 or 2.5 mg/kg body weight. The authors did not report the radiochemical purity of ^{14}C-atrazine. It was reported that due to its low solubility in ethanol, ^{14}C-atrazine tended to flake off the skin surface (with no chance in being absorbed) resulting consistently in high variability in different parameters measured between the treated animals. Data obtained from the high-dose group was thus not considered in determining the potential risk of dermal exposure to atrazine.

Results reported here indicate that there are no major differences between the male and female rats in the dermal absorption of ^{14}C-atrazine at different time points examined. Total absorption was reported to be approximately 70 percent of the dose within 72 hours after treatment resulting in an absorption half-life in boh sexes of 40.9 hours. The amount of radioactivity present in different tissues assayed varied with the time of exposure. Muscle contained the highest quantities (as percent of the dose) possibly due to its size (muscle accounts for approximately 45 to 50 percent of the total animal weight) and did not appear to have high affinity for atrazine-derived radioactivity. Based on tissue concentration (ug/g tissue) liver, kidney, and large intestine appeared to have the highest concentrations especially at the 24-, 48-, and 72-hour time points. These results also indicate that absorption is still occurring at 72 hours since concentrations in blood, liver, and kidney are still increasing.

Most of the radioactivity cleared from the tissues was excreted in the urine and to a lesser extent in the feces within 72 hours, accounting for approximately 46 percent of the applied dose (in urine and feces combined) or 55 percent of the absorbed dose. These results suggest that the half-life for total body clearance (male and female) is long, possibly exceeding 72 hours.

Although the authors reportedly analyzed whole urine for metabolites, a number of questions are raised by this reviewer as to the methods used and the way the results were reported for the following points:

1. Why was mostly urine from the high-dose group male and female animals analyzed in spite of the fact that data from the high-dose group were considered highly unreliable?

2. Why did the authors not isolate, quantitate, and identify any ^{14}C-atrazine metabolites. The conversion (by

oxidative hydrolysis) of atrazine and/or metabolites to cyanuric acid does not provide much information as to the nature of the atrazine-derived radioactivity present in the urine.

3. The authors reported that approximately 82 percent of the radioactivity in the urine was converted to cyanuric acid and 5 percent to methyl cyanuric acid. Where is the remainding 13 percent of the radioactivity? Neither in the Methods nor in the Results section is there any mention of methyl cyanuric acid being used as standard on TLC plates. How did the authors decide that 5 percent of the radioactivity was methyl cyanuric acid? For the particular solvent system used, Rf values should have been calculated for each peak observed.

Conclusions

Data reported here indicate that approximately 70 percent of a dose of ^{14}C-atrazine applied to the skin of male or female rats (at 0.25 mg/kg) is absorbed through the skin within 72 hours with an estimated half-life for absorption of 40.9 hours. Most of the absorbed dose is excreted mainly in the urine and to a lesser extent in the feces within 72 hours. Based on concentrations of atrazine-derived radioactivity in the tissues absorption continues beyond 72 hours after application.

Classification:

The present study is classified as Core-Supplementary based on deficiencies discussed above. The study, however, can be upgraded to Core-Guideline classification when the authors provide us with (1) radiochemical purity of ^{14}C-atrazine, and (2) supply more information on atrazine residues in urine.

TABLE 1 BALANCE DATA FOR PRE-EVALUATION HIGH DOSE SHOWING PERCENTAGE RECOVERY OF APPLIED RADIOACTIVITY 72 HOURS AFTER DOSING

Fraction	72 Hour High Female
Urine	46.13
Feces	10.76
Volatiles	.20
CO_2	.14
Skin Wash	15.12
Dissolved Skin	.29
Tissues	6.20
Cage Wash	13.33
Carcass	13.59
	105.76

TABLE 9. BALANCE DATA FOR LOW AND HIGH DOSES OF ^{14}C-ATRAZINE SHOWING PERCENTAGE RECOVERY OF APPLIED RADIOACTIVITY 72 HOURS AFTER DOSING

Fraction	Low Dose		High Dose	
	Males	Females	*Males	Females
Urine	33.39 ± 4.6	35.82 ± 5.7	41.17 ± 2.65	43.42 ± 5.8
Feces	11.9 ± 1.7	10.52 ± 1.7	11.13 ± 1	11.85 ± 3.5
Skin Wash	27.62 ± 8.2	29.16 ± 3.1	12.6 ± 6.5	12.06 ± 5.04
Dissolved Skin	.84 ± .17	.63 ± .04	.24 ± .06	2.68 ± .39
Tissues	5.45 ± 1.0	6.64 ± .6	3.62 ± .74	3.8 ± .58
Blood	.86 ± .17	1.05 ± .12	.67 ± .22	.8 ± .2
Carcass	12.4 ± 4	13.87 ± 1.2	9.05 ± 1.6	10.22 ± 1.6
Cage Wash	7.33 ± 5.0	5.34 ± .53	15.95 ± 6	16.41 ± 3.07
	99.79	103.03	94.43	101.24

*Average percentage is calculated from 5 dosed rats.

TABLE 3 AVERAGE AMOUNT OF DOSE RECOVERED IN THE URINE AND AND FECES AT COLLECTED TIME POINTS DURING THE BALANCE STUDY PHASE

Urine

	Male Low Dose	Female Low Dose	Male High Dose	Female High Dose
24 hours	12.26 ± 2.95	15.10 ± 1.09	20.04 ± 3.75	22.22 ± 8.06
48 hours	12.31 ± 1.98	11.80 ± .84	12.69 ± 1.78	14.38 ± 2.53
72 hours	8.80 ± .48	8.88 ± 1.01	8.16 ± 1.51	6.81 ± .44

Feces

	Male Low Dose	Female Low Dose	Male High Dose	Female High Dose
24 hours	3.29 ± 1.04	3.05 ± 1.19	4.56 ± .55	3.90 ± 1.56
48 hours	4.72 ± 1.24	3.97 ± 1.35	3.99 ± 1.18	4.87 ± 1.92
72 hours	3.92 ± 1.15	3.48 ± 1.05	2.57 ± .57	3.06 ± 1.53

TABLE 4 AMOUNT OF DOSE REMAINING ON SKIN AT TIME POINTS CORRESPONDING TO THE KINETIC PHASE AND BALANCE PHASE OF THE STUDY

Hours From Treatment to Sacrifice	Low Dose		High Dose	
	Males	Females	Males	Females
0	106.12 ± 3.5	103.83 ± 1.02	99.68 ± 3.11	101.05 ± .66
2	96.93 ± 2.4	88.11 ± 5.8	75.48 ± 14	64.93 ± 18
4	88.59 ± 1.4	86.7 ± 7.2	77.36 ± 14.5	62.35 ± 13
8	86.79 ± 9	75.35 ± 5.35	58.96 ± 7.6	47.92 ± 23
24	67.9 ± 6.5	67.3 ± 10.5	17.66 ± 5	16.46 ± 2
48	41.67 ± 14.5	42.0 ± 7.5	28.0 ± 15	13.92 ± 6
72	28.46 ± 8.2	29.78 ± 3.0	12.8 ± 6.6	14.74 ± 5.3

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