# Metabolism of $^{14}$C-Labeled Hexazinone in the Rat

Robert C. Rhodes* and Richard A. Jewell[1]

The active ingredient in Velpar weed killer is 3-cyclohexyl-6-(dimethylamino)-1-methyl-1,3,5-triazine-2,4(1*H*,3*H*)-dione (hexazinone). When a rat was given a single oral dose of radiolabeled hexazinone by intragastric intubation, the radioactivity was eliminated within 72 h in the urine (61%) and feces (32%). Trace amounts of radioactivity were also found in the gastrointestinal tract (0.06%) and expired air (0.08%). No radioactivity (<0.01%) was detected in the body tissues. The major metabolites in both urine and feces were 3-(4-hydroxycyclohexyl)-6-(dimethylamino)-1-methyl-1,3,5-triazine-2,4(1*H*,3*H*)-dione and 3-(4-hydroxycyclohexyl)-6-(methylamino)-1-methyl-1,3,5-triazine-2,4(1*H*,3*H*)-dione.

Velpar weed killer (E. I. du Pont de Nemours & Co., Inc.) is a highly effective herbicide for the control of many annual and perennial broadleafed weeds, grasses, herbaceous vines, and woody plants. Velpar is registered in the United States and many foreign countries for noncrop weed control, including drainage ditch banks. An Experimental Use Permit and Temporary Residue Tolerance of 0.2 ppm were granted in 1977 by EPA for evaluation of Velpar for controlling weeds in sugar cane. An Experimental Use Permit for aquatic weed control tests has been granted by EPA. Velpar is also registered in certain foreign countries for weed control in oil palm, rubber, and tea. The active ingredient of Velpar is hexazinone [3-cyclohexyl-6-(dimethylamino)-1-methyl-1,3,5-triazine-2,4-(1*H*,3*H*)-dione (DPX-3674)].

Studies on the fate of Velpar in soil (Rhodes, 1980a) and in water and fish (Rhodes, 1980b) have also been reported. This paper describes metabolism studies with $^{14}$C-labeled hexazinone in a rat. Details on the preparation of $^{14}$C-labeled hexazinone and its metabolites are also presented.

## EQUIPMENT AND METHODS

Radioactivity in liquid samples (urine, blood, and sodium hydroxide) was determined by direct counting using a Nuclear Chicago liquid scintillation spectrometer (Model 6801). Solid samples were analyzed by combustion in a Packard Model 305 sample oxidizer, followed by liquid scintillation counting.

Chromatographic separations were made on TLC plates (250 μm silica gel 60F-254, E. M. Laboratories, Inc.) and developed in a 9:1 (v/v) mixture of ethyl acetate and methanol or a 9:1 (v/v) mixture of chloroform and methanol. The location of the $^{14}$C-labeled materials on the TLC plates was detected using a Varian-Aerograph/Berthold Model 6000-2 automatic/integrating TLC radioscanner.

All mass spectra were obtained on a Dupont Model 21-492 high-resolution mass spectrometer.

## EXPERIMENTAL SECTION

**Synthesis of $^{14}$C-Labeled Hexazinone.** Radiolabeled hexazinone was prepared according to the sequence of reactions shown in Figure 1. The individual reaction steps were conducted as follows.

*Reaction 1.* Methyl 4-cyclohexyl-1-thioallophanimidate (Lin, 1975) (3.8 g) in 20 mL of benzene was placed in a 100-mL round-bottom flask fitted with a thermometer, dropping funnel, drying tube, and magnetic stirrer, and the solution was cooled to 6 °C. A solution containing 179.1 mg (36.5 mCi) of methyl chloroformate-$^{14}$*C* (carbonyl) (New England Nuclear) and 1.77 g of nonradiolabeled methyl chloroformate in 25 mL of benzene (at 6 °C) was added to the flask. Triethylamine, 2.5 mL in 5 mL of benzene, was added dropwise, with stirring, over a 20-min period. The mixture was heated at 50 °C for 2 h and stirred overnight at 25 °C. Water (15 mL) was added, and after ca. 2 min stirring, the aqueous phase was removed with a pipet and discarded. The benzene solution was dried over sodium sulfate and taken to dryness under a slow stream of nitrogen to give methyl 4-cyclohexyl-*N*-(methoxycarbonyl)-1-thioallophanimidate-$^{14}$*C* (I).

*Reaction 2.* A sodium methoxide solution (0.5 M, 35.6 mL) was added to I, and the mixture was heated under reflux for 2 h. The solvent was evaporated under a stream of nitrogen and the residue was triturated twice with 80-mL portions of diethyl ether. The ether was removed by filtration, and the solids were dried with a stream of nitrogen to give sodium 3-cyclohexyl-6-methylthio-1,3,5-triazine-2,4(1*H*,3*H*)-dione-$^{14}$*C* (II).

*Reaction 3.* Acetonitrile (40 mL) and methyl iodide (1.22 mL) were added to II, and the mixture was heated under reflux for 12 h. The solvent was evaporated with a stream of nitrogen and the residue was triturated with 80 mL of water. The water was removed by filtration and the solid, 1-methyl-3-cyclohexyl-6-methylthio-1,3,5-triazine-2,4(1*H*,3*H*)-dione-$^{14}$*C* (III, mp 135–136 °C) was recrystallized from 5 mL of 2-propanol with 80 mL of ice water added to the cooled slurry to complete precipitation. The solid was thoroughly dried at 50 °C under a slow stream of nitrogen.

*Reaction 4.* A 1 M solution of dimethylamine in anhydrous THF (60 mL) was added to III and the mixture was stirred overnight at 25 °C. The solvent was evaporated under a stream of nitrogen to give 3.10 g of solid. The residue was dissolved in 4 mL of ethyl acetate and placed on a 4.5 × 14 cm column of "SilicAR" CC-7 Special (Mallinckrodt Chemical Works) and eluted from the column with ethyl acetate. The solvent was evaporated with nitrogen to give 2.19 g of [$^{14}$C]hexazinone (mp 106–107 °C, sp act., 7.11 μCi/mg). Overall yield was 42.5% based on radioactivity.

**Chemical Purity.** A 2.20-mg aliquot was dissolved in 1.0 mL of acetone and 5-, 10-, 50-, and 100-μL aliquots were applied on a 20 × 20 cm silica gel plate. The plate was developed for 15 cm with a 9:1 (v/v) mixture of ethyl acetate and methanol.

The plate was examined under a short-wavelength (254 μm) ultraviolet light. All aliquots gave a single dark spot on a light-green fluorescent background at a $R_f$ value of 0.40, the same as a reference standard of hexazinone. Since

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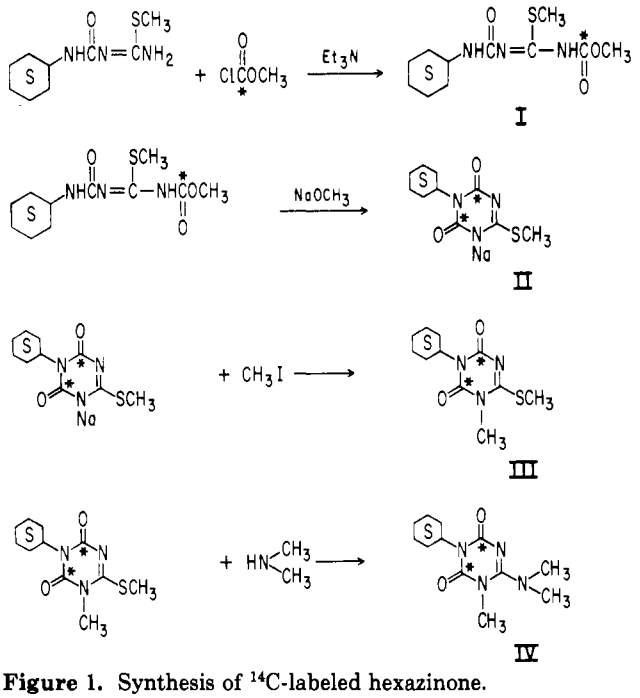


Figure 1. Synthesis of $^{14}C$-labeled hexazinone.

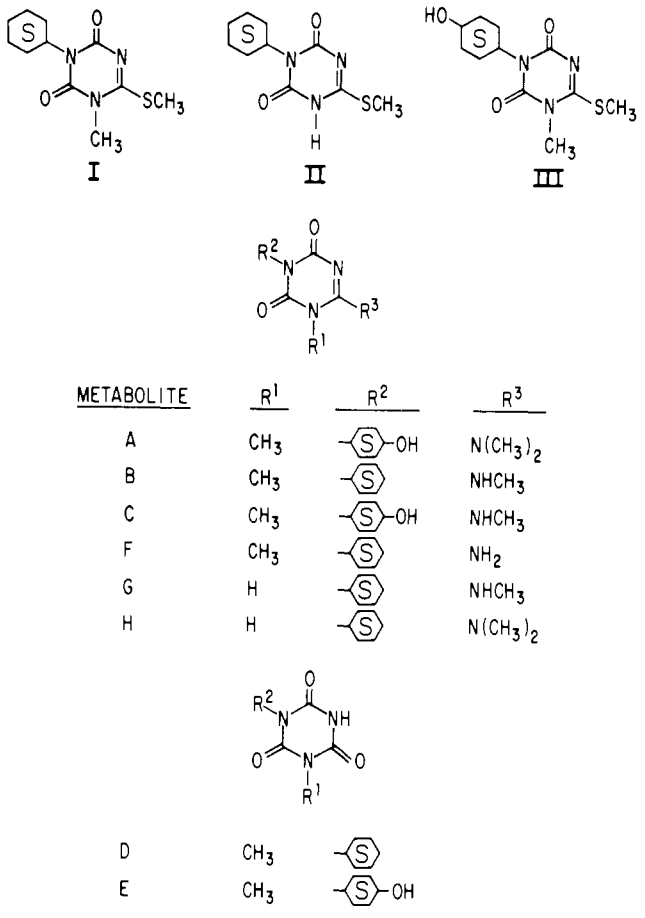
the 5-μL spots were clearly visible and only a single spot was observed with the 100-μL aliquot, the chemical purity was estimated to be greater than 95% based on UV absorbing contaminants.

**Radiochemical Purity.** The location of radioactive materials on the above plate was determined using the TLC radioscanner with integrator. The radiochemical purity, calculated from the integrated areas, was greater than 99%. The procedure above was repeated using a mixture of chloroform and methanol (9:1, v/v) as the development solvent. This TLC radioscan also showed that the radiochemical purity was greater than 99%.

**Synthesis of Metabolites.** The intermediate 3-cyclohexyl-6-methylthio-*s*-triazine-2,4(1*H*,3*H*)-diones (compounds I and II, Figure 2), and 3-cyclohexyl-6-amino-*s*-triazine-2,4(1H,3H)-diones B, G and H (Figure 2) were prepared according to U.S. Patent No. 3 902 887 (Lin, 1975).

Compound III was prepared by the following procedure. Gaseous HCl (43.8 g) was added to a mixture of acetic anhydride (113 mL) and glacial acetic acid (800 mL). To this mixture, 4-hydroxycyclohexylamine (115.2 g, K&K Chemical Co., distilled at 123 °C/15 torr) was slowly added. After heating at reflux for 16 h, the solution was evaporated to a residue under vacuum at 65 °C. Trituration with ether, filtration, and washing with ether gave 147 g of 4-acetoxycyclohexylamine hydrochloride (mp 175–179 °C). This product (115 g) was added to 1 L of xylene at reflux, and phosgene was slowly added until all solid had dissolved. The reaction mixture was cooled and evaporated to a residue under vacuum at 65 °C. Distillation of the residue (97 °C/0.5 torr) gave 93.1 g of 4-acetoxycyclohexylisocyanate. A mixture of this isocyanate (45.8 g) and 1-carbomethoxy-2-methyl-2-thiopseudourea (37.1 g; Lin, 1975) in $CH_2Cl_2$ (200 mL) was stirred for 16 h at ambient temperature. After removal of the solvent by evaporation under vacuum at 45 °C, the residue was triturated with *n*-butylchloride to yield 42.2 g of compound III (mp 150–155 °C).

Compounds F, A, and C were prepared from intermediates I or III. To 5.0 g of I in 50 mL of methanol was added 5 mL of concentrated ammonium hydroxide. After stirring about 16 h at 23 °C, the slurry was filtered, washed



| METABOLITE | $R^1$ | $R^2$ | $R^3$ |
|---|---|---|---|
| A | $CH_3$ | -S-OH | $N(CH_3)_2$ |
| B | $CH_3$ | -S- | $NHCH_3$ |
| C | $CH_3$ | -S-OH | $NHCH_3$ |
| F | $CH_3$ | -S- | $NH_2$ |
| G | H | -S- | $NHCH_3$ |
| H | H | -S- | $N(CH_3)_2$ |
| D | $CH_3$ | -S- | |
| E | $CH_3$ | -S-OH | |

Figure 2. Synthesis and structures of hexazinone degradation products. Metabolite A, 3-(4-hydroxycyclohexyl)-6-(dimethylamino)-1-methyl-1,3,5-triazine-2,4(1*H*,3*H*)-dione; metabolite B, 3-cyclohexyl-6-(methylamino)-1-methyl-1,3,5-triazine-2,4-(1*H*,3*H*)-dione; metabolite C, 3-(4-hydroxycyclohexyl)-6-(methylamino)-1-methyl-1,3,5-triazine-2,4(1*H*,3*H*)-dione; metabolite D, 3-cyclohexyl-1-methyl-1,3,5-triazine-2,4,6(1*H*,3*H*,5*H*)-trione; metabolite E, 3-(4-hydroxycyclohexyl)-1-methyl-1,3,5-triazine-2,4,6(1*H*,3*H*,5*H*)-trione; metabolite F, 3-cyclohexyl-6-amino-1-methyl-1,3,5-triazine-2,4(1*H*,3*H*)-dione; metabolite G, 3-cyclohexyl-6-(methylamino)-1,3,5-triazine-2,4(1*H*,3*H*)-dione; metabolite H, 3-cyclohexyl-6-(dimethylamino)-1,3,5-triazine-2,4(1*H*,3*H*)-dione.

with methanol, and dried to give 3.0 g of F (mp 286–287 °C). Similarly, 5.0 g of III in THF treated with 25% dimethylamine gave, after evaporating the reaction mixture, triturating with *n*-butylchloride, and recrystallizing from ethyl acetate, 3.6 g of A (mp 191.5–193 °C). In the same manner, 2.0 g of III in MeOH treated with 40% methylamine gave 1.3 g of C (mp 319–321 °C).

Compounds G and H were prepared from intermediate II. A solution of 2.0 g of II in 20 mL of THF was saturated with methylamine and heated in a bomb at 100 °C overnight. The mixture was evaporated to give 1.4 g of G (d. 250). Reaction between II and dimethylamine under the same conditions gave H (d. 280).

Compounds D and E were prepared according to the following example. A mixture of 1.5 L of 1 N HCl and 150 g of I was heated at reflux for 3 h. The slurry was cooled to room temperature, filtered, washed with water, and dried to give 124.9 g of D (mp 209–212 °C). Similarly, 2.0 g of III gave 1.2 g of E (mp 232–235 °C).

**Animal Study.** A male Charles River-CD rat no. 154846, weighing 201 g, was given a diet composed of ground Purina Laboratory Chow to which 1% corn oil and 2500 ppm unlabeled hexazinone had been added. Seventeen days later, when the animal weighed 390 g, it was

given 2 mL of an aqueous solution containing 18.3 mg (130 μCi) of [$^{14}$C]hexazinone by intragastric intubation and was immediately placed in a glass metabolism unit (Stanford Glassblowing Labs, Inc.) through which 500 mL/min of dried and $CO_2$ free air was drawn. The effluent air was passed through a 4 N sodium hydroxide trap to remove respiratory [$^{14}$C]carbon dioxide, then through a cupric oxide hot tube (500 °C) to oxidize other respiratory organic $^{14}$C compounds to $^{14}CO_2$, and finally through another 4 N sodium hydroxide trap to collect possible $^{14}CO_2$ products in the catalytic oxidation furnace. The NaOH in the caustic traps was changed twice daily, frozen, and retained for total $^{14}$C analysis. Three 24-h samples of urine and feces were collected and frozen for analysis.

After 72 h, the animal, weighing 376 g, was lightly anesthetized with chloroform and blood was removed from the heart. The animal was sacrificed and all organs and tissues were surgically remmoved, immediately frozen, and maintained frozen until analyzed.

Aliquots of the gas trap samples, urine, and blood samples were analyzed for total $^{14}$C by direct liquid scintillation counting. Tissue and feces samples were homogenized with water in a blender and freeze-dried. Aliquots of the freeze-dried tissue and feces samples were analyzed for $^{14}$C residues by combustion–liquid scintillation counting.

**Metabolite Identification.** Urine (10 mL) from the 0–24-h collection was adjusted to pH 5 with 1 N $H_2SO_4$, 2 g of β-glucuronidase–aryl sulfatase enzyme preparation ($4 \times 10^5$ Fishman units/g, Sigma Chemical Co.) was added and the resulting solution was incubated for 48 h at 37 °C. Aliquots (1 mL) of the resulting urine, and of fresh urine, were applied as a streak to silica gel TLC plates and the plates developed for 15 cm in ethyl acetate–methanol (9:1, v/v). The TLC radioscans showed the same four radioactive bands, none of which corresponded to hexazinone, in both fresh and hydrolyzed urine. The silica corresponding to each of the radioactive areas was scraped from the plate, and the $^{14}$C materials were removed from the silica gel by successive washings with 25-mL portions of methanol (three times) and chloroform (three times). The washings from each radioactive area were combined and the volumes of the four resulting solutions were reduced to ca. 0.5 mL in vacuo at 50 °C. These solutions were then applied on silica gel TLC plates and developed three times for 15 cm in chloroform–methanol (9:1, v/v) and radioscans were obtained. The $^{14}$C-labeled compounds were removed from the plates by the procedure described above. An aliquot (100 μL) of each resulting solution was counted in 15 mL of Aquasol scintillation solution to determine the total $^{14}$C in each fraction. The remainder of each solution (400 μL) was used to obtain a mass spectrum of the isolated metabolite.

An aliquot of the 0–24-h feces sample (freeze-dried), equivalent to 80% of the total 0–24-h collection, was extracted four times with 25-mL portions of methylene chloride using ultrasonication. The combined extract was centrifuged to remove solids and the volume of the extract reduced to 5.0 mL in vacuo at room temperature. A 50-μL aliquot of the extract was counted in Aquasol scintillation solution to determine percent radioactivity extracted (of the total $^{14}$C determined by combustion analysis) and a second aliquot (250 μL) of the extract was analyzed by the TLC method described above.

## RESULTS AND DISCUSSION

When a male rat which had been preconditioned on 2500 ppm of hexazinone in the diet was treated by intragastric intubation with 18.3 mg (130 μCi) of radiolabeled hexazinone, almost all of the radiolabeled material was rapidly eliminated from the animal body. Approximately 61% was recovered from the urine and 32% from the feces within 72 h (Table I). Trace amounts of radioactivity were also found in the gastrointestinal tract (0.06%) and expired air (0.08%). No radioactivity (<0.01%) was detected in the body tissues after 72 h. Total recovery of radioactivity was 93.3% of the applied dose.

TLC analyses of urine samples, both before and after enzymatic hydrolysis (β-glucuronidase–arylsulfatase), show no hexazinone (<1%). In fact, TLC radioscans of the urine, before and after hydrolysis, were qualitatively the same, indicating very little or no conjugation of the hexazinone metabolites. Figure 3 shows the TLC radioscan for fresh rat urine (0–24 h). Percent radioactivity for all identified metabolites is given in Table II. The major

Table I. [$^{14}$C]Hexazinone Metabolism Study in the Rat (Total Dose, 130 μCi)

| radioactivity detected 72 h after dose | % of original $^{14}$C |
|---|---|
| body tissues | |
| heart | <0.01 |
| kidney | <0.01 |
| spleen | <0.01 |
| lung | <0.01 |
| brain | <0.01 |
| muscle | <0.01 |
| liver | <0.01 |
| testes | <0.01 |
| blood | <0.01 |
| G.I. tract | 0.06 |
| gas traps (expired air) | |
| prefurnace | 0.08 |
| postfurnace | <0.01 |
| urine | |
| 0-24 h | 57.2 |
| 24-48 h | 3.14 |
| 48-72 | 0.42 |
| feces | |
| 0-24 h | 23.21 |
| 24-48 h | 8.03 |
| 48-72 h | 1.11 |
| total recov | 93.3 |

Table II. Analysis of [$^{14}$C]Hexazinone Rat Urine

| compd[d] | % of $^{14}$C | $R_f$[a] | $R_f$[b] |
|---|---|---|---|
| A | 46.8 | 0.17, 0.27[c] | 0.57 |
| B | 11.5 | 0.37 | 0.70 |
| C | 39.3 | 0.17, 0.27[c] | 0.27 |
| D | 1.5 | 0.73 | 0.77 |
| E | <1.0 | 0.77 | 0.40 |
| F | 0.5 | 0.37 | 0.53 |
| G | 0.4 | 0.37 | 0.60 |
| hexazinone | <1.0 | 0.40 | 0.80 |

[a] Developed to 15 cm in a 9:1 mixture of ethyl acetate–methanol. [b] Developed three times to 15 cm in a 9:1 mixture of chloroform–methanol. [c] Two radioactive areas detected due to cis-trans isomers. [d] See Figure 2 structures.

Table III. Analysis of [$^{14}$C]Hexazinone Rat Feces

| compd[a] | % of extracted $^{14}$C (0-24 h) |
|---|---|
| A | 26.3 |
| B | 2.1 |
| C | 55.2 |
| D | 0.4 |
| E | 6.9 |
| F | 6.6 |
| G | 2.5 |
| hexazinone | <1.0 |

[a] 90.2% of $^{14}$C extracted from the feces.

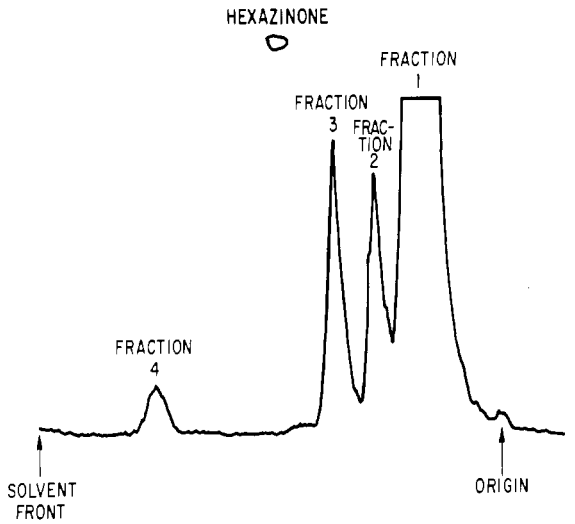



**Figure 3.** Radioscan of TLC plate (9:1, ethyl acetate–methanol) for rat urine.

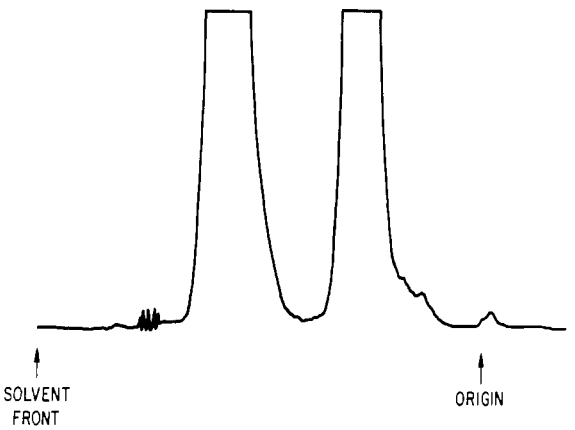



**Figure 4.** Fraction 1 radioscan of TLC plate (9:1, chloroform–methanol, 3×) for rat urine.

urinary metabolites were the 4-hydroxycyclohexyl compounds, A (46.8%) and C (39.3%) (Figure 2). Both fractions I and II, when chromatographed in a second solvent system [three times in chloroform–methanol (9:1, v/v)], showed two radioactive areas each on the TLC plates (Figure 4). The mass spectra of the compounds with the lower $R_f$ value (0.27) from each fraction in this separation were identical (compound C) and the mass spectra of the compounds at the higher $R_f$ values (0.57) were also identical (compound A). The two pairs of compounds, each with identical mass spectra but different $R_f$ values in the first TLC system are presumably due to separation of the corresponding cis–trans isomers of the respective 4-hydroxycyclohexyl compounds (A and C). The structural assignment for all urinary metabolites has been confirmed by mass spectroscopy using reference standards.

The major metabolites in the feces were also found to be compounds A and C. The percent of radioactivity for all fecal metabolites, which were identified by TLC $R_f$ values, is given in Table III. The percent of total $^{14}C$ extracted from the 0–24 h feces sample was 90.2%.

## CONCLUSIONS

This $^{14}C$ study has confirmed that hexazinone is metabolized and eliminated rapidly from a mammalian system as was also shown in nonradioactive rat and dog feeding studies (Pease and Holt, 1977). The primary metabolic degradation has been shown to be hydroxylation of the cyclohexyl ring and monodemethylation of the dimethylamino group. Comparison of the mass spectra of synthesized metabolites with those isolated from the rat have confirmed identities. Details on the mass spectral interpretation and confirmation will be presented at a later time (Reiser et al., 1979).

## ACKNOWLEDGMENT

The author gratefully acknowledges H. Sherman of Haskell Laboratory for the animal dosing and R. W. Reiser for the mass spectral work. Appreciation is also expressed to N. L. Redfearn and K. Lin for syntheses and assistance in describing the preparation of [$^{14}C$]hexazinone and metabolites.

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# Studies with $^{14}C$-Labeled Hexazinone in Water and Bluegill Sunfish

Robert C. Rhodes

$^{14}C$ residues in bluegill sunfish exposed to water containing [$^{14}C$]hexazinone at 0.01 and 1.0 ppm for 4 weeks were found to plateau after 1–2 weeks of exposure. A maximum accumulation factor of 5–7 was found in the viscera at both exposure levels. Following the 4-week exposure period, the fish were transferred to fresh water for a 2-week depuration period. After 1 week in fresh water the $^{14}C$-residue levels decreased by greater than 90% and no detectable $^{14}C$ residues remained in the fish tissue at the end of the 2-week period. No effects on the fish were noted during the course of the experiment.

Application of Velpar weed killer is a highly effective treatment for the control of many annual and perennial broadleafed weeds, grasses, herbaceous vines, and woody plants. The active ingredient of Velpar is 3-cyclohexyl-6-(dimethylamino)-1-methyl-1,3,5-triazine-2,4(1*H*,3*H*)-dione (hexazinone).

Metabolism of [$^{14}C$]hexazinone in the rat (Rhodes and Jewell, 1980) and studies on the fate of hexazinone in soil (Rhodes, 1980) have been reported.

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