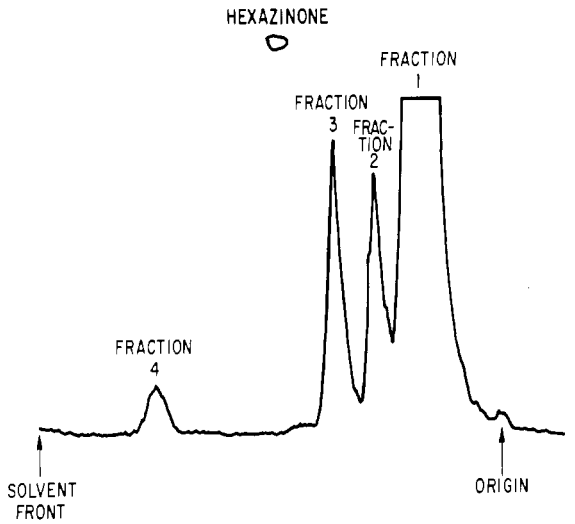

Figure 3. Radioscan of TLC plate (9:1, ethyl acetate–methanol) for rat urine.

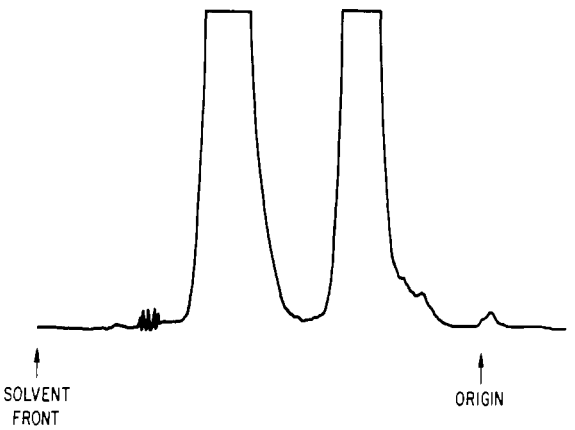

Figure 4. Fraction 1 radioscan of TLC plate (9:1, chloroform–methanol, 3×) for rat urine.

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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Received for review March 10, 1978. Accepted October 4, 1979.

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

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^{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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0021-8561/80/1428-0306$01.00/0

This paper described studies on the photodegradation of hexazinone in aqueous solutions and residue studies with bluegill sunfish exposed to [^{14}C]hexazinone.

EQUIPMENT AND METHODS

Radioactivity in aqueous samples 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 thin-layer chromatographic (TLC) plates (250 μm silica gel, 60 F-254, E. M. Laboratories, Inc.) 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 Du Pont Model 21-492 high-resolution mass spectrometer.

The preparation of [^{14}C]hexazinone and the reference standards of hexazinone degradation products used in this study have been previously described (Rhodes and Jewell, 1980).

EXPERIMENTAL SECTION. PART I

Hydrolysis Study. The stability of [^{14}C]hexazinone toward hydrolysis was evaluated under the following conditions: 5990 ppm (2 lb/40 gal) in 0.05 M, pH 5, 7, and 9 aqueous buffers (Fisher Scientific Co., Fairlawn, NJ) at 15 °C; 5 ppm in 0.05 M, pH 4,7, and 9 aqueous buffers at 15 °C; and 5 ppm in 0.05 M, pH 7 buffer at 25 and 37 °C. All solutions were kept in the dark in screw-capped bottles in thermostated, constant temperature baths.

Aliquots of each solution were taken for analysis at 1-week intervals for 8 weeks. The aliquots were applied to 250 μm silica gel TLC plates and the TLC plates were developed to 15 cm in a mixture of ethyl acetate and methanol (9:1, v/v). The plates were air-dried and scanned to determine the location of the radioactive materials.

Under all conditions, only one radioactive compound was detected by TLC at R_f 0.40 corresponding to hexazinone. The area of silica gel containing this radioactive material from the 8-week sample of the 5 ppm solution in pH 7 buffer at 15 °C was removed from the TLC plate. The gel was washed three times with 20-mL portions of methanol to remove the radioactivity from the silica gel. The methanol washings were combined and the volume of the resulting solution was reduced to ca. 0.5 mL with a stream of nitrogen at room temperature. A mass spectrum of the isolated ^{14}C material was obtained.

Laboratory Photodegradation Studies in Water. The photodegradation of [^{14}C]hexazinone in aqueous solutions was studied in the laboratory using an artificial light source, similar to that described by Hirt et al. (1960), to approximate "natural sunlight". The light source was constructed (Shoplite Co., Inc., Nutley, NJ) as a bank of 12 lamps, mounted under a polished aluminum cover. The bank of lamps consisted of six fluorescent sunlamps (FS20, Westinghouse Co.) alternated with six fluorescent blacklamps (F20T12/FL, General Electric Co.) with a spacing of 5 cm between lamp centers. The light unit was mounted on a stand over a table.

The ultraviolet light intensity (microwatts per square centimeter) of the light source was measured with a Black-Ray ultraviolet intensity meter (Ultra-Violet Products, Inc., Model J-221). The average ultraviolet light intensity (300–400 nm, peak sensitivity ca. 365 nm), measured 15 cm from the lamps, was ca. 1200 μW/cm^2, which is equivalent to about half of the intensity of typical summer sunshine at noon (Hirt et al., 1960).

Four aqueous solutions (listed below), each containing 5 ppm [^{14}C]hexazinone, were used for this study. The solutions, in 400-mL jacketed beakers, were placed under the center of the light source at a distance of 15 cm from the lights. Water (15 ± 1 °C) from a thermostated bath (Haake Instruments, Inc., Saddlebrook, NJ, Model FK-2) was continuously circulated through the beaker jackets. The depth of the solutions (200 mL) was ca. 5 cm. Solutions containing 5 ppm [^{14}C]hexazinone were made from each of the following water samples: (1) distilled water (pH 6.7), (2) standard reference water (pH 8.1) (Freeman, 1953), (3) distilled water with anthraquinone (20 mg/L) added as a photoinitiator, and (4) distilled water with riboflavin (20 mg/L) added as a photoinitiator.

Standard reference water is a laboratory-made water containing appropriate amounts of specific inorganic salts. It is reported to be the equivalent of an average or typical surface water in the United States (Freeman, 1953).

These solutions were then exposed to light from the lamps for a period of 8 weeks for solutions 1 and 2 and 5 weeks for the remainder of the solutions. Water losses from the aqueous solutions due to evaporation were corrected by addition of distilled water, as needed. Aliquots of each sample were taken for analysis every 7 days.

Duplicate samples of each solution, in glass-stoppered bottles, were placed in the dark at 15 ± 1 °C and were used as control samples.

At each sampling, an aliquot (1 mL) of each solution was counted to determine total ^{14}C in the solution. A second 1-mL aliquot of each solution was applied directly to a 250-μm silica gel TLC plate, and the plates were developed 15 cm in a mixture of ethyl acetate and methanol (9:1 v/v). The location of the ^{14}C compounds on the TLC plates was determined by scanning the plates with a radioscanner. The area of silica gel, containing each ^{14}C compound, was scraped into a scintillation vial containing 3.5 mL of water. Scintillation solution (11.5 mL) was added to each vial, and the vials were shaken. The resulting suspensions were counted.

Degradation of [^{14}C]Hexazinone in Aqueous Solutions Exposed to Sunlight. The photodegradation of [^{14}C]hexazinone in water was studied under summer sunlight in Wilmington, DE. Solutions containing 5 ppm [^{14}C]hexazinone were made from each of the following water samples: (1) standard reference water, pH 8.1, (2) standard reference water containing 20 ppm riboflavin, (3) Brandywine River water, and (4) Brandywine River water with 2.5 cm of bottom sediments. The Brandywine River is a northern Delaware river from which the city of Wilmington obtains its water supply. The river water and river bottom sediments were taken from the river at Brecks Mill.

Each solution (200 mL, 5 cm deep) was placed in a 400-mL jacketed beaker and placed on a table in a location which received full-day sunlight. Specially constructed stands with quartz tops were placed above each of the beakers to prevent rain and debris from entering the solutions. The quartz tops were mounted on legs 15 cm high, leaving a 3-cm clearance between the top of the beaker and the quartz plate to allow free air circulation above the solution surface. Water (14 ± 1 °C) from a thermostated bath was continuously circulated through the beaker jackets for the duration of the experiment. The temperature of one of the solutions (standard reference water) was monitored continuously using a recording thermometer (Taylor Instrument Co., Asheville, NC). The temperature

Table I. Analysis of Aqueous Solutions of [^{14}C]Hexazinone Exposed to UV Light

distribution (%) of radioactivity applied to TLC plates																
distilled water										standard reference water							
exposure, weeks										exposure, weeks							
1	2	3	4	5	6	7	8	^{14}C compd[a]	R_f[b]	1	2	3	4	5	6	7	8
	1	1	2	3	3	4	5	origin	0.00		2	4	5	6	8	10	10
	1	1	2	3	3	4	5	A	0.17		7	6	9	11	14	18	23
100	95	94	91	90	87	87	82	B, H, hexazinone	0.40	100	87	82	79	73	68	64	55
	2	2	3	3	4	5	5	C	0.27		2	3	4	6	6	8	7
	1	1	1		1		1	D	0.90		1	1	2	2	2		3
			1	1	2		2	E	0.80		1	4	1	2	2		2

distribution (%) of radioactivity applied to TLC plates											
distilled water + anthraquinone							distilled water + riboflavin				
exposure, weeks							exposure, weeks				
1	2	3	4	5	^{14}C compd	R_f[b]	1	2	3	4	5
1	1	2	3	4	origin	0.00	8	15	20	25	19
1	2	3	3	4	A	0.17	13	19	23	23	34
84	80	76	73	68	B, H, hexazinone	0.40	53	43	37	34	31
4	4	6	8	11	C	0.27	20	15	11	10	8
1	2		3	4	D	0.90	1	1		2	2
9	11	13	10	9	E	0.80	5	7	9	6	6

[a] See Figure 1 for structures. [b] TLC system: ethyl acetate-methanol (9:1, v/v).

Table II. Analysis of Aqueous Solutions of [^{14}C]Hexazinone Exposed to Natural Sunlight

distribution (%) of radioactivity applied to TLC plates											
standard, reference water							standard reference water + riboflavin				
exposure, weeks							exposure, weeks				
1	2	3	4	5	^{14}C compd[a]	R_f[b]	1	2	3	4	5
	1	1	4		origin	0.00	1	1	7	4	
	6	3	6	6	A + C	0.40	4	6	6	11	13
3	4	11	11	17	B	0.55	6	7	10	13	16
	2	4	6	8	H	0.70	9	10	11	12	15
97	87	81	73	69	D, hexazinone	0.80	80	76	66	60	56

distribution (%) of radioactivity applied to TLC plates											
Brandywine River water							Brandywine River water and sediment				
exposure, weeks							exposure, weeks				
1	2	3	4	5	^{14}C compd	R_f[b]	1	2	3	4	5
		1	7	9	origin	0.00					2
6		5	14	16	A + C	0.40			1	7	6
3	14	17	14	18	B	0.55	6	9	12	10	11
	5	7	9	8	H	0.70		5	6	7	10
91	81	70	56	49	D, hexazinone	0.80	94	86	81	76	71

[a] See Figure 1 for structures. [b] TLC system: chloroform-methanol (9:1, v/v).

in the monitored solution was 15.5 ± 1.5 °C throughout the test period.

At five weekly intervals, an aliquot of each sample was analyzed for total ^{14}C in the solution and a second aliquot was analyzed for [^{14}C]hexazinone and [^{14}C] degradation products by the methods previously described. However, for TLC, a mixture of chloroform and methanol (9:1 v/v) was used as the developing solvent. The radiolabeled material with R_f values at 0.40, 0.55, and 0.70 were isolated from the TLC plates as before, and a mass spectrum was obtained on each isolated fraction.

At the end of the 5-week test period the sediments were removed from the natural river water by filtration. The sediments were freeze-dried and duplicate 1-g aliquots of the dried sediments were analyzed for ^{14}C content by combustion in a Packard Model 305 sample oxidizer, followed by liquid scintillation counting. A 10-g sample of the sediments was extracted three times with 25-mL portions of 10% water in acetone in a blender for 10 min. The extracts were combined, and the volume of the extract was reduced to ca. 2 mL in vacuo at 50 °C. The solution was quantitatively transferred to a 3-mL volumetric flask and made to volume with acetone. A 50-μL aliquot was counted to determine the ^{14}C content of the extract. A 250-μL aliquot was analyzed for hexazinone and breakdown products by the TLC procedure above.

RESULTS AND DISCUSSION. PART I

Dilute aqueous solutions of hexazinone in the dark are stable (<1% decomposition) for at least 5–8 weeks at pH 5, 7, and 9 and at temperatures of 15, 25 and 37 °C as indicated by TLC and confirmed by GC/MS. In distilled water exposed to "artificial sunlight", hexazinone is slowly degraded (ca. 10% in 5 weeks) (Table I). On the basis of 5-week data, the amount of decomposition under both artificial and natural sunlight is about three times greater in standard reference water and about four to seven times greater in natural river water or in distilled water containing 20 ppm of riboflavin. The degree of decomposition in distilled water containing 20 ppm of anthraquinone

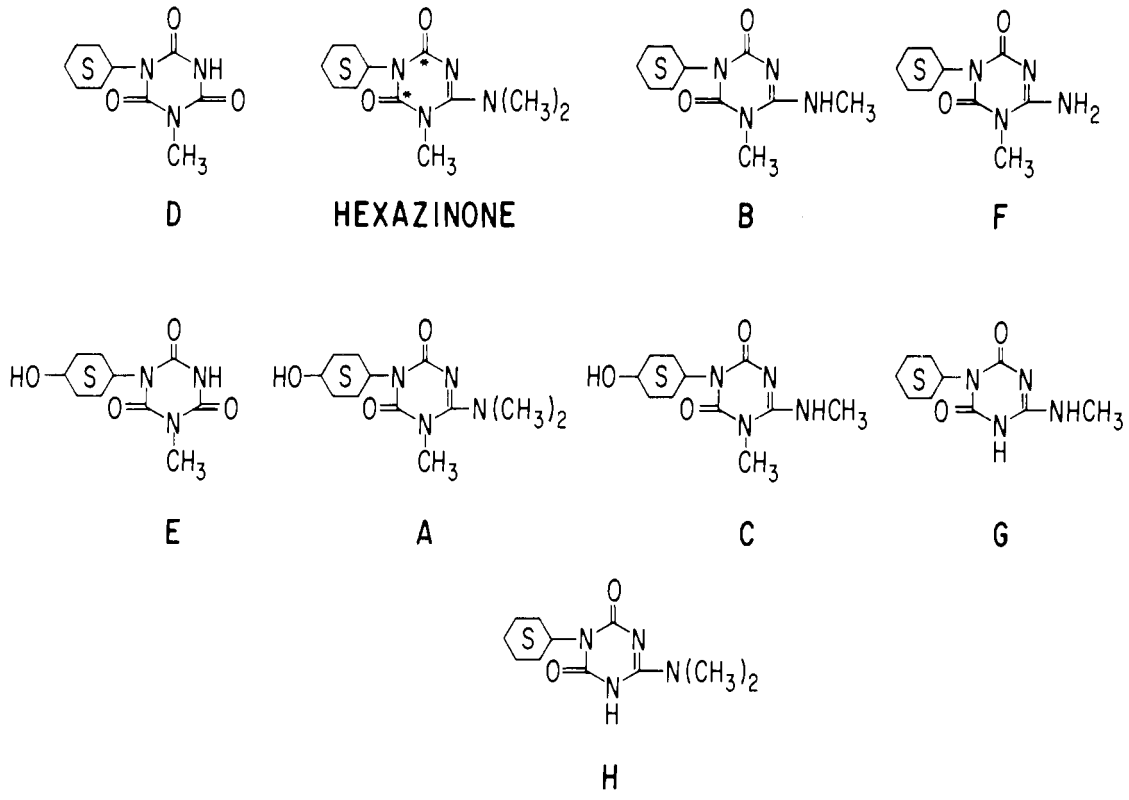

Figure 1. Structures of hexazinone degradation products. Metabolite A, 3-(4-hydroxycyclohexyl)-6-(dimethylamino)-1-methyl-1,3,5-triazine-2,4(1*H*,3*H*)-dione; metabolite B, 3-cyclohexyl-6-(methylamino)-1-methyl-1,3,5-triazine-2,4(1*H*,3*H*)-dione; metabolite C, 3-(4-hydroxycyclohexyl)-6-(methylamino)-1-methyl-1,3,5-triazine-2,4(1*H*,3*H*)-dione; 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; and metabolite H, 3-cyclohexyl-6-(dimethylamino)-1,3,5-triazine-2,4(1*H*,3*H*)-dione. (*) Denotes position of label.

under artificial lights and in natural river water with bottom sediments is about three times the amount in distilled water (Tables I and II). At the end of the 5-week exposure period, about 10% of the ^{14}C in the river water with sediments was found in the sediments. Extraction of the sediments removed 67% of the ^{14}C. TLC analysis of the extract showed about the same distribution of ^{14}C compounds that was found in the river water, e.g., 70% hexazinone in the sediments and 65% in the water.

The major routes of photodegradation involve demethylation to give compounds B and H (Figure 1) and hydroxylation to give compound A. The structures of these major degradation products were confirmed by mass spectroscopy (Reiser et al., 1979).

Mass spectroscopic confirmation of the identity of compounds C, D, and E was not obtained. In previous studies, C, D, and E were shown by mass spectroscopic analysis to be metabolites of hexazinone in rat urine (Rhodes and Jewell, 1980). The TLC separation data in Tables I and II show small amounts of D and E in the laboratory studies.

EXPERIMENTAL SECTION. PART II

Fish Exposure to [^{14}C]Hexazinone. The exposure portion of the test was conducted by Bionomics, Inc. (Wareham, MA) using a modification of a continuous-flow proportional dilution apparatus (Mount and Brungs, 1967) which provides for the automatic intermittent introduction of the test material and diluent water into the test chamber. Three 30-L glass aquariums (units A, B, and C) were utilized in the experiment. One hundred bluegill sunfish (*Lepomis macrochirus*) having a mean weight of 3.4 g and a mean length of 63 mm were placed into each unit. Aerated well water (pH 7.1; total hardness, 40 mg/L as $CaCO_3$; dissolved oxygen, >5.0 mg/L; temperature, 20 ± 1.0 °C) was provided to each unit at a flow rate of 5 L/h.

Table III. [^{14}C]Hexazinone Concentration in Water (ppm)[a]

exposure period	0.01-ppm test	1.0-ppm test
3 day	0.013	1.3
1 week	0.013	1.3
2 week	0.010	1.0
3 week	0.011	1.1
4 week	0.011	1.1

[a] Radioactivity was greater than 99% hexazinone at all sampling periods.

The stock solution used for the 10 μg/L (0.01 ppm) [^{14}C]hexazinone exposure experiment was prepared by mixing 0.108 g (768 μCi) of [^{14}C]hexazinone (sp act., 7.11 μCi/mg) with 100 mL of water. Another stock solution for the 1000 μg/L (1.0 ppm) experiment was prepared by mixing 10.8 g (768 μCi) of [^{14}C]hexazinone (sp act., 0.0711 μCi/mg) with 1 L of water. While the fish in unit A served as a control, fish in unit B were continuously exposed to [^{14}C]hexazinone at a concentration of 10 μg/L, and those in unit C were continuously exposed at 1000 μg/L. The mechanical dilution apparatus previously mentioned was used to establish and maintain the desired chemical concentration. Fish were fed a dry pelleted ration ad libitum each day.

Analysis. Water and fish were sampled from each unit on days 0, 3, 7, 14, 21, and 28 of the exposure period. Fish remaining in units B and C after the 28-day exposure were transferred to clean flowing water for a 14-day recovery period. Fish were sampled 1, 3, 7, and 14 days after transfer into the fresh water. Water samples (50 mL) were taken from units B and C on all sampling days during the

Table IV. Total ^{14}C Residue in Bluegill Sunfish (ppm)

	0.01 ppm [^{14}C]hexazinone in water			1.0 ppm [^{14}C]hexazinone in water		
exposure period	carcass	liver	viscera	carcass	liver	viscera
3 days	0.01	0.02	0.04	1.0	1.3	2.0
1 week	0.01	0.03	0.07	1.3	1.6	5.3
2 weeks	0.02	0.03	0.07	2.1	5.0	6.7
3 weeks	0.02	0.02	0.04	2.0	2.7	5.5
4 weeks	0.02	0.02	0.05	1.0	1.9	4.6
			Withdrawal Phase			
1 day	0.01	0.02	0.03	0.5	2.1	3.7
3 day	<0.01	<0.01	<0.01	<0.1	0.2	0.6
1 week	<0.01	<0.01	<0.01	<0.1	<0.1	0.2
2 week	<0.01	<0.01	<0.01	<0.1	<0.1	<0.1

exposure period. At each sampling interval (both exposure and withdrawal), five fish were removed from each unit and eviscerated. The carcasses, viscera, and livers of fish from each exposure level were pooled, and all samples were maintained frozen until analyzed.

The carcasses (edible tissue) and viscera from each sampling interval were blended separately with water and freeze-dried. Aliquots of the freeze-dried samples were analyzed for total ^{14}C residues by combustion–liquid scintillation counting. The combined livers from each sampling were analyzed for total ^{14}C content as a single sample which was combusted in toto. Water (3.5 mL) from each sampling was directly analyzed for ^{14}C concentration by liquid scintillation counting.

Identification of Radioactive Residues. The treated water remaining after the ^{14}C concentration analysis (46.5 mL) for each sampling interval was analyzed to determine the chemical identity of the radioactive material. The volume of each water sample was reduced to 1 mL in vacuo at 50 °C. An aliquot (0.1 mL) of each concentrated solution was applied to silica gel TLC plates and developed for 15 cm in a 9:1 (v/v) mixture of chloroform and methanol. The developed plates were air-dried and scanned to determine the location of the radioactive material.

Carcasses of fish from the 28-day exposure, both levels, were combined for analysis. The fish (65.0 g, fresh weight) were extracted three times by macerating with 100-mL portions of chloroform. The chloroform extracts were combined, and the solution was taken to dryness in vacuo at ambient temperature. The residue was dissolved in 50 mL of acetonitrile. The acetonitrile was washed three times with 50-mL portions of hexane, and the hexane, after counting and showing that it contained no radioactivity, was discarded. Water (50 mL) was added to the acetonitrile solution and the volume of the resulting solution was reduced to about 40 mL in vacuo at ambient temperature. The aqueous solution was washed two times with 50-mL portions of hexane and the hexane discarded. The aqueous solution was then extracted two times with 50-mL portions of chloroform. The chloroform extracts were combined, and the volume was reduced to about 1 mL in vacuo. The concentrated extract was quantitatively transferred to a 2-mL volumetric flask and made to volume with chloroform. A 50-μL aliquot of the extract was counted to determine the radioactivity in the extract. The extracted tissue was analyzed by combustion–liquid scintillation counting to determine the unextracted radioactivity.

A 0.5-mL aliquot of the extract was applied to a TLC plate and the plate developed as before. The TLC scan showed two radioactive areas at R_f 0.40 and 0.80. The area of silica gel containing each ^{14}C compound was removed from the plate and placed into a scintillation vial containing 3.5 mL of water. Scintillation solution (11.5 mL) was added, the vial was shaken, and the resulting suspensions were counted.

RESULTS AND DISCUSSION. PART II

Hexazinone was stable in the aqueous solutions for the 4-week test period. The exact concentration of [^{14}C]hexazinone in the water at each sampling period is given in Table III. No decomposition (<1%) was observed at all sampling times for both the 0.01 and 1.0 ppm treatments. No fish mortality was observed. Fish exposed to 0.01 ppm and 1.0 ppm [^{14}C]hexazinone in water behaved normally and appeared to be in excellent physical condition during both the 28 days of exposure and the 14 days of the recovery phase.

The ^{14}C residues (calculated as ppm hexazinone) in bluegill sunfish exposed to water treated with 0.01 and 1.0 ppm hexazinone are shown in Table IV. The residue levels in all tissues were low and were found to plateau after 1–2 weeks of exposure. The maximum accumulation factor (^{14}C residue in fish tissue/concentration in water) of ca. 5–7 occurred in the viscera at both exposure levels. The accumulation factors in the liver and carcass were 3–5 and 2, respectively. The residue levels in all tissues decreased by greater than 90% after a 1-week withdrawal period in fresh water, and no detectable radiolabeled residues remained after a 2-week withdrawal period.

The major portion of the radioactivity extracted from the fish was unchanged hexazinone. The extraction procedure removed 80% of the ^{14}C from the fish tissue. TLC analysis showed that 91% of the extracted ^{14}C was unchanged hexazinone and 9% was 3-(4-hydroxycyclohexyl)-6-dimethylamino-1-methyl-1,3,5-triazine-2,4-(1*H*,3*H*)-dione, a major urinary metabolite (metabolite A) of hexazinone in the rat (Rhodes and Jewell, 1980).

ACKNOWLEDGMENT

The author gratefully acknowledges R. W. Reiser for obtaining and interpreting the mass spectra of the photodegradation products and G. A. Smith for his skillful laboratory assistance.

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Received for review March 10, 1978. Accepted October 4, 1979.