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DATA EVALUATION RECORD

STUDY 3

CHEM 128969 Triasulfuron §165-4

FORMULATION--00--ACTIVE INGREDIENT

STUDY ID 42782005
Fackler, P.H. 1991a. CGA-131036 (phenyl ring label) - bioconcentration and elimination of 14C-residues by bluegill (Lepomis macrochirus). SLI Report No. 90-11-3549; Sponsor Protocol/Project No. 28-90. Unpublished study performed by Springborn Laboratories, Inc., Wareham, MA, and Ciba-Geigy Corporation, Greensboro, NC; and submitted by Ciba-Geigy Corporation, Greensboro, NC.

Phelps, W. 1993. Response to review of Bioaccumulation in Fish studies. This response to the reviewed studies 42782004/-05 was requested by the EFGWB during the review process. Letter of W. Phelps (CIBA) dated 12/8/93.

DIRECT REVIEW TIME = 31

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CONCLUSIONS:

a. Administrative

The original review of the 165-4 studies questioned the lack of analytical sensitivity due to the low specific activity of the radiolabelled test substance as a result of "dilution" with non-radioactive test material.

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The EFGWB reviewer contacted CIBA for further comments/data. The Branch considers that the response of CIBA is appropriate and, therefore, the two bioaccumulation in fish studies are now ACCEPTABLE.

The EFGWB had originally waived the 165-4 data requirement based on the low octanol/water partition coefficient of triasulfuron. However, the Ecological Effects Branch requested the studies based on the persistence of triasulfuron in aqueous media.

b. Scientific

Parent triasulfuron does not have the tendency to bioaccumulate in fish. Bluegill sunfish exposed to a concentration of triasulfuron of 1.4 mg/L for 28 days in a flow-through system showed bioaccumulation factors of 0.46x for edible tissues, 2.6x for inedible tissues, and 1.3x for whole fish. Parent triasulfuron was the major and/or major residue detected in extractable fractions of 14 and 28 days exposure.

METHODOLOGY:

Bluegill sunfish (Lepomis macrochirus; mean length 55 mm, mean weight 2.15 g) were held in culture tanks on a 16-hour photoperiod for ≥14 days prior to the initiation of the study. Flow-through aquatic exposure systems were prepared using two 100-L glass aquaria. Aerated well water (pH 6.8-7.5, dissolved oxygen content 68-87% of saturation, total hardness 32-35 mg/L as $CaCO_3$, and alkalinity 18-24 mg/L as $CaCO_3$) was added to each aquarium at a rate of approximately 8.3 turnovers per day. The aquaria were immersed in a water bath and maintained at 17 ± 1 C.

One aquarium was treated at a nominal 1.4 mg/L with phenyl ring-labeled [^{14}C]triasulfuron (1-[2-(2-chloroethoxy)phenylsulfonyl]-3-(4-methoxy-6-methyl-1,3,5-triazin-2-yl)urea; CGA-131036; radiochemical purity 98.5%, specific activity 30.5 uCi/mg, Ciba-Geigy) dissolved in acetone; the remaining aquarium was treated with pesticide-free acetone and served as a control. After an equilibration period of 7 days, the triasulfuron concentration in the aquarium water was confirmed by LSC, and 175 fish were transferred to each aquarium. During the exposure period, water (5 mL) and fish (5) were collected from each tank at 1, 3, 7, 10, 14, 21, 25, and 28 days; additional fish (25) were collected at 14 and 28 days for residue analysis. Following the 28-day exposure period, 35 fish were transferred from the treated aquarium into an aquarium containing pesticide-free water; the water flow rate was approximately 8.3 turnovers per day. During the 14-day depuration period, water (5 mL) and fish (5) were collected at 1, 3, 7, 10, and 14 days.

The five fish collected at each sampling interval were dissected into fillet (body, skin, muscle, and skeleton) and visceral (fins, head, and internal organs) tissues, air-dried for ≥24 hours, and analyzed for total radioactivity using LSC following combustion. It was reported that "a 0.50 gram tissue sample, 60 cpm above background,

with an 85% counting efficiency has a minimal detectable limit of 0.53 mg/kg." A portion of the fish collected at 28 days were filleted, and the edible tissues were combined (three sets of approximately 10 fish each) and homogenized with dry ice in an analytical mill. The tissues were sequentially extracted with hexane and acetonitrile by vortexing; between extractions, the samples were centrifuged and the supernatant decanted. The hexane extracts were evaporated to dryness under a stream of nitrogen; the acetonitrile extracts were filtered through a 0.7 mm glass fiber filter, then evaporated to dryness. The resulting residues were redissolved in scintillation fluid (Monophase-S), and the solutions were analyzed using LSC to determine the distribution of nonpolar and polar [^{14}C]residues. The extracted tissue was analyzed for unextracted radioactivity using LSC following combustion.

Aliquots of the aquaria water samples were analyzed for total radioactivity using LSC; "a 5.00 mL water sample, 50 cpm above background with a 90% counting efficiency has a minimal detectable limit of 0.044 mg/L.". Additional aliquots of the water were analyzed by HPLC using a Phenomenex Ultramex C-18 column eluted with acetonitrile:water (1:1, acidified to pH 3 with H_2PO_4); the column was equipped with variable wavelength detection.

The remaining fish collected at 14 and 28 days during exposure were separated into fillet and visceral tissues, frozen, and shipped to the analytical laboratory on dry ice. The fish were received cold but not frozen at the analytical laboratory.

These tissues were extracted and analyzed for [^{14}C]metabolites according to the specific schemes presented in Figures 4-7. In general, portions (7-27 g) of the tissues were homogenized in an analytical mill and extracted twice with acetonitrile:water (8:2, v:v) by stirring for 30-45 minutes. Between extractions, the samples were centrifuged, and the supernatants were decanted and filtered. The two extracts from each tissue were combined, and aliquots were analyzed by LSC. Additional aliquots of the extracts were concentrated (method not given), and the concentrate was partitioned three times with methylene chloride. The methylene chloride fractions were combined, and aliquots were analyzed by LSC.

Aliquots of the methylene chloride extracts from the nonedible fish tissues were analyzed by two-dimensional TLC on silica gel plates developed in ethyl acetate:methylene chloride:glacial acetic acid (50:50:1) in the first direction and acetonitrile:ethyl acetate:formic acid (150:50:1.5) in the second direction. The samples were cochromatographed with unlabeled reference standards, which were visualized by UV light. [^{14}C]Compounds on the plates were located by autoradiography. Aliquots of the methylene chloride extracts from the nonedible fish tissue were also analyzed by reverse-phase HPLC using a Phenomenex Lichrosorp 5 RP18 column eluted with 35% acetonitrile in acidified water (0.0125% TFA); the column

was equipped with UV and radioactive flow detection. The extracted tissues were analyzed by LSC following combustion.

The methylene chloride extracts from the edible tissue were purified using a silica SepPak cartridge; the column was eluted twice with methanol. The methanol eluates were combined, and aliquots were analyzed by two-dimensional TLC as previously described. The methylene chloride-extracted acetonitrile:water solution was purified on a C-18 Bond Elut cartridge; the cartridge was eluted twice with methanol. Aliquots of the methanol eluates were analyzed by two-dimensional TLC and HPLC as previously described. The extracted tissue was analyzed by LSC following combustion.

CIBA conducted further analyses of Day 14 and 28 tissues and presented the results on 12/8/93.

DATA SUMMARY:

[^{14}C]Triasulfuron residues accumulated in bluegill sunfish that were continuously exposed to phenyl ring-labeled [^{14}C]triasulfuron (1-[2-(2-chloroethoxy)phenylsulfonyl]-3-(4-methoxy-6-methyl-1,3,5-triazin-2-yl)urea; CGA-131036; radiochemical purity 98.5%) at approximately 1.4 mg/L for 28 days in a flow-through aquarium system. The bioconcentration factors were 0.46x for edible tissues, 2.6x for inedible tissues, and 1.3x for whole fish. Maximum concentrations of total [^{14}C]residues were 8.6 mg/kg in edible tissues, 12 mg/kg in nonedible tissues, and 9.9 mg/kg in whole fish (all at 10 days; Table II).

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was isolated from the inedible fish tissues.

All detectable [^{14}C]residues were extracted from the fish tissues with acetonitrile:water (8:2, v:v) by stirring (Tables II and IV). In the 14- and 28-day edible tissue extract (0.18-0.23 ppm, Table I), [^{14}C]triasulfuron was the predominant extractable [^{14}C]residue; quantitative data were not provided. In the 14-day inedible tissue extract, [^{14}C]triasulfuron was the only compound detected and was 6.46 ppm (Table VII). In the 28-day inedible tissue extract, [^{14}C]triasulfuron was 1.39 ppm, unidentified polar [^{14}C]residues were 0.38 ppm, and uncharacterized nonpolar [^{14}C]residues (quadrant III) were 0.30 ppm.

Recovery of [^{14}C]residues from the fish tissues during depuration was erratic because of high detection limits (Table II). In the edible tissues, [^{14}C]residues were detected at 0.11-0.21 ppm in three of five fish analyzed at 10 days; [^{14}C]residues not detected (detection limit up to 0.33 ppm) in any fish at 14 days. In the inedible tissues, [^{14}C]residues were detected at 0.17 ppm in one of five fish analyzed at 10 days; [^{14}C]residues not detected (detection limit up to 0.39 ppm) in any fish at 14 days.

During the 30-day exposure period, the concentration of total [^{14}C]residues in the water ranged from 1.4 to 1.7 mg/L (Table II). In the 10- and 25-day water samples, [^{14}C]triasulfuron was the predominant [^{14}C]compound. Throughout the study, the temperature of the treated, untreated and depuration aquaria water was 17 $\pm$ 1 C, the pH ranged from 7.2-7.8, and the dissolved oxygen content was 8.5-9.3 mg/L (Table I).

COMMENTS:

a. To study 42782005

1. The analytical method appeared to be too insensitive to accurately determine the accumulation of [^{14}C]residues in the fish. For LSC, it was reported that "a 0.50 gram tissue sample, 60 cpm above background with an 85% counting efficiency has a minimal detectable limit of 0.53 mg/kg." For HPLC, the detection limits were not reported. For the initial HPLC analyses (nonpolar and polar residue distribution), the [^{14}C]residue detection limits appeared to range up to 0.63 and 0.69 mg/kg in the edible and inedible tissues, respectively (day 3 of exposure and day 3 of depuration, respectively; Table II). For the HPLC analyses conducted to characterize [^{14}C]metabolites, the [^{14}C]residue detection limits were "twice background dpms". The radioactive test material may have been over-diluted with unlabeled triasulfuron; the study author reported that radioactive triasulfuron comprised 0.334% of the test material and that counting times were as high as 100 minutes.

2. Uncharacterized nonpolar [^{14}C]residues migrating to TLC quadrant III were present at up to 0.30 ppm in the inedible fish tissue. No apparent attempt was made to characterize these residues. Subdivision N guidelines require that [^{14}C]compounds in the fish tissues present at $\geq$0.05 ppm be identified.

3. The frozen storage conditions for the water and fish samples prior to analysis, including the temperature and storage intervals, were not provided. The study author also reported that the samples thawed during shipment to the analytical laboratory.

4. The concentrations of [^{14}C]residues in the whole fish were not determined directly, but were calculated from the concentrations of [^{14}C]residues in the edible and inedible tissues.

5. The study author stated that the inedible tissue from one of the five 7-day exposure sample, which contained [^{14}C]residues

at 67 mg/kg, "may have been contaminated during sample collection and analysis."

6. No mortality or abnormal behavior was observed in the control and treated fish during the entire study.

7. Throughout the study, fish were fed pelleted food daily at a rate of 1-2% of their total biomass, except 24 hours prior to testing.

8. A proposed metabolic pathway for triasulfuron has been detailed in Figure 20.

b. To CIBA's response to above comments (12/8/93)

The reviewer accepts the arguments presented by CIBA in their letter dated 12/8/93. Copy of the response is attached to this review. Data presented indicate the low potential of trisulfuron for bioaccumulating in bluegill sunfish.

Table II. ^{14}C-Residue Concentrations, Calculated as CGA-131036 (Phenyl Ring Label), Measured in Fish Tissue Portions and Exposure Solution Water During 28 Days of Exposure of Bluegill (*Lepomis macrochirus*) to CGA-131036 (Phenyl Ring Label) and During 14 Days of Depuration.

Test Day	Water Concentration (mg/L)	Tissue Concentration (mg/kg)[b]		
		Edible (Muscle)	Non-edible (Carcass/ Viscera)	Whole Body[a]
Exposure				
1	1.4	< 0.09	0.17	0.12
	1.5	< 0.24	< 0.34	0.28
	1.4	< 0.15	< 0.23	0.19
		< 0.14	< 0.22	0.17
		< 0.16	< 0.24	0.19
Mean	1.4	0.16	0.24	0.19
S.D.	0.1	0.05	0.06	0.06
3	1.5	3.6	6.8	4.8
	1.4	< 0.63	0.92	0.77
	1.4	0.35	5.4	2.4
		0.27	0.34	0.29
		< 0.25	< 0.41	0.31
Mean	1.4	1.0	2.8	1.7
S.D.	0.1	1.4	3.1	1.9
7	1.5	< 0.16	1.3	0.59
	1.7	0.49	67[c]	29
	1.6	0.14	0.26	0.18
		< 0.23	0.54	0.37
		0.32	0.60	0.44
Mean	1.6	0.27	14	6.1
S.D.	0.1	0.14	30	13

Table II. Continued.

Test Day	Water Concentration (mg/L)	Tissue Concentration (mg/kg)[b]		
		Edible (Muscle)	Non-edible (Carcass/ Viscera)	Whole Body[a]
Exposure				
25	1.7	< 0.11	0.43	0.23
	1.6	< 0.32	6.3	3.0
	1.6	< 0.25	1.0	0.60
		< 0.16	0.84	0.44
		< 0.23	0.41	0.31
Mean	1.6	0.21	1.8	0.91
S.D.	< 0.1	0.08	2.5	1.2
28	1.7	< 0.15	0.89	0.45
	1.7	< 0.33	< 0.46	0.39
	1.6	3.4	3.3	3.4
		< 0.53	7.5	3.8
		0.54	8.4	4.3
Mean	1.7	0.99	4.1	2.4
S.D.	< 0.1	1.36	3.7	1.9
Depuration				
1	< 0.049	< 0.082	0.31	NA
	< 0.049	0.13	0.82	0.38
	< 0.049	< 0.077	0.41	NA
		< 0.21	< 0.35	NA
		< 0.080	0.43	NA

Table IV. **^{14}C-Residue Concentrations, Calculated as CGA-131036 (Phenyl Ring Label), Measured in Extractions of Edible (Muscle) Tissue of Bluegill (Lepomis macrochirus) After 28 Days of Continuous Aqueous Exposure to CGA-131036 (Phenyl Ring Label).**

	Replicate 1	Replicate 2	Replicate 3
Grams edible tissue extracted	10.4372	10.5708	10.0952
µg hexane extractable	0	0	0
µg acetonitrile extractable	1.6	0.91	1.2
µg non-extractable	0	0	0

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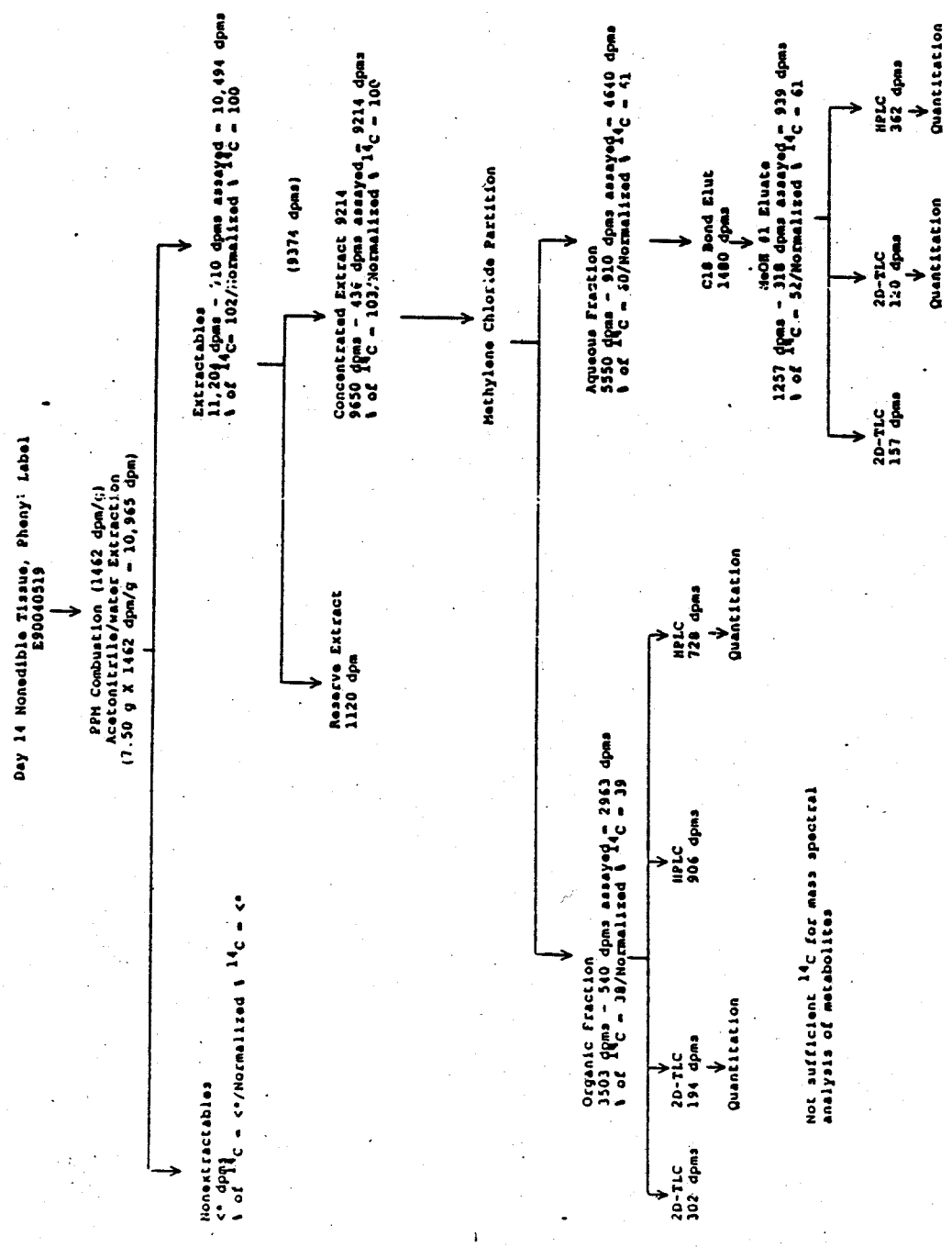
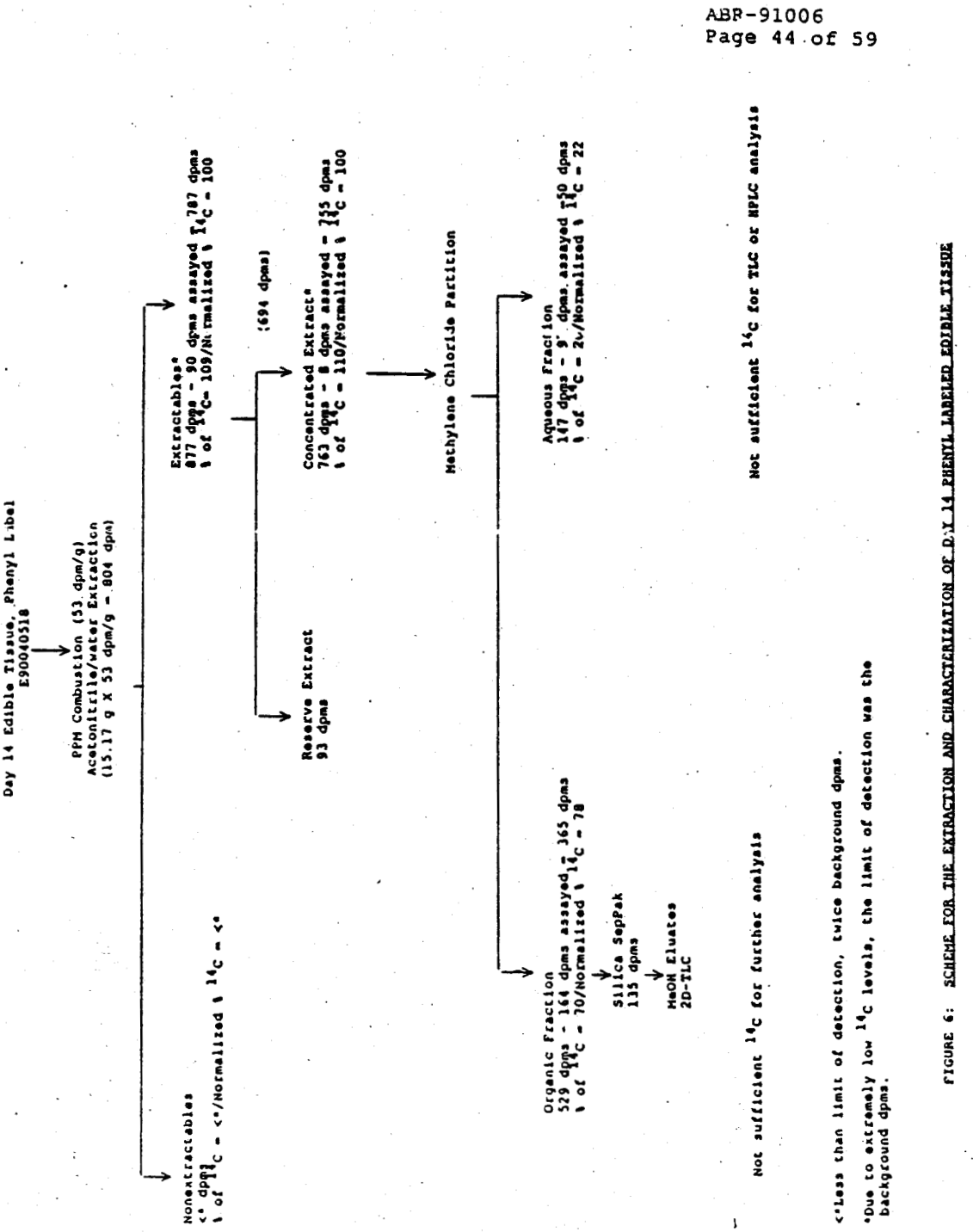


FIGURE 4: SCHEME FOR THE EXTRACTION AND CHARACTERIZATION OF DAY 14 PHENYL LABELED NONEDIBLE TISSUE



*Less than limit of detection, twice background dpms.

*Due to extremely low ^{14}C levels, the limit of detection was the background dpms.

FIGURE 6: SCHEME FOR THE EXTRACTION AND CHARACTERIZATION OF D:X 14 PHENYL LABELED EDIBLE TISSUE

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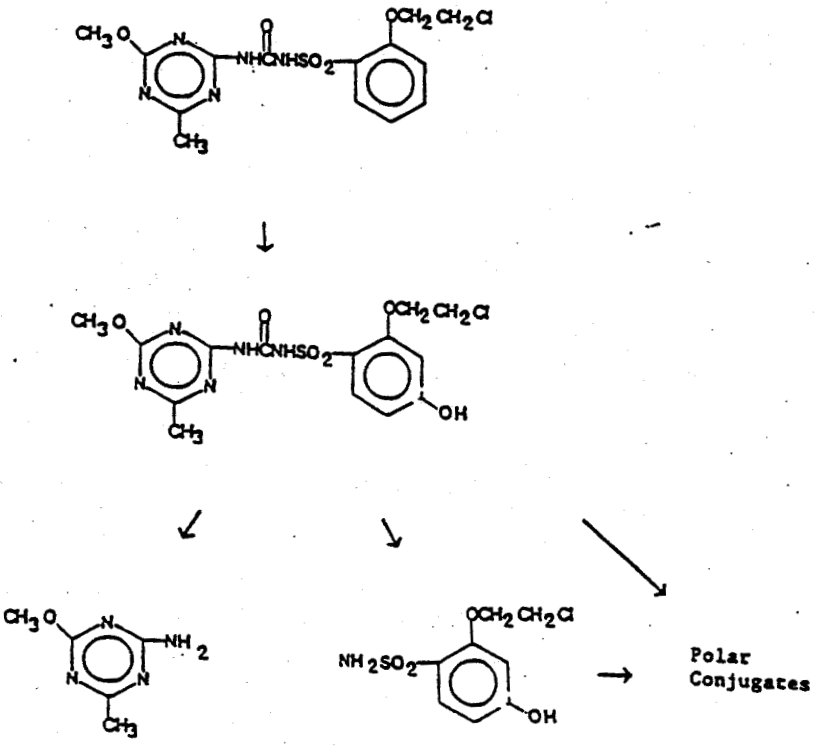

FIGURE 20: PROPOSED METABOLIC PATHWAY

3.0 RESULTS

3.1 Evaluation of the Bioconcentration Factor

Analyses of the test solution samples removed on each of the seven days before the exposure was initiated resulted in measured concentrations which averaged 111% of the nominal concentration. Based on these results, the exposure was initiated.

The bioconcentration study exposing bluegill to a nominal concentration of 1.4 mg/L and a solvent control solution was terminated after 28 days of exposure and 14 days of depuration. Throughout the study, undissolved test material was observed in the dilution system and the test aquaria. As undissolved material was observed, it was removed. During both the exposure and depuration periods, no mortality occurred among the test organisms. In general, the fish appeared healthy and exhibited normal behavior throughout the study.

During the 42 day study, the measured water quality parameters varied minimally between test aquaria and remained within acceptable ranges for the maintenance of bluegill. Results of the water quality measurements made in the test aquaria during this test are summarized in Table I. Continuous monitoring of the temperature in the treatment aquaria throughout the

pretreatment and day 28 of the exposure period established that no detectable levels were present. (Table III).

Based on a mean measured concentration of 1.5 mg/L (± 0.1) in the exposure solution during the 28-day exposure period and the mean steady state tissue concentrations, bioconcentration factors were calculated. The bioconcentration factors (BCF) of CGA-131036 (phenyl ring label) in bluegill tissue were 0.46 X in edible tissue (muscle); 2.6 X in non-edible tissue (viscera and carcass); and 1.3 X in whole body tissue.

Predicted bioconcentration factors for edible, non-edible and whole body fish tissue were also calculated. Equation 1 (see Page 19) was utilized in a computer program to determine the uptake (K_u) and the depuration constant (K_d) for each tissue type. K_u/K_d established the predicted BCF.

Tissue type	K_u/K_d	Predicted BCF	Measured BCF
Edible	0.35/0.70	0.50	0.46
Non-edible	1.6/0.56	2.8	2.6
Whole Body	0.84/0.59	1.4	1.3

Results of the polar (acetonitrile) solvent and the nonpolar (hexane) solvent extractions of edible tissue samples revealed that 1.2 μg of the ^{14}C-residues accumulated in the exposure were extractable with acetonitrile. None were extractable with hexane and none could be quantified as non-extractable. Due to the extremely low bioconcentration factors, insufficient ^{14}C-residues were available in tissue removed from day 28 of exposure.

3.2 Depuration Phase

Half-life (50% elimination) of the ^{14}C-residues present in the whole body tissue of bluegill on the last day of exposure could not be determined due to the extremely low bioconcentration

RESULTS AND DISCUSSION

Tissue Residues: The radioactive levels in the edible and nonedible tissues are given in Table I. Regardless of the label, ppm equivalents to ^{14}C-CGA-131036 were very low. For the phenyl labeled edible tissues, the ppm values were 0.23 ppm and 0.18 ppm at days 14 and 28, respectively. Similarly, the ppm levels in the triazine labeled edible tissues were 0.33 at day 14 and were less than the limit of detection at day 28.

The phenyl labeled nonedible tissues contained 6.46 ppm equivalents at day 14.. After 28 days, the ppm values decreased to 2.55 ppm. Nonedible tissues with the triazine ring label had similar ppm levels of 2.79 and 3.00 on days 14 and 28, respectively.

Characterization of Extractable Metabolites: The schemes for the extraction, separation, and characterization of metabolites are outlined in Figures 4-10. Acetonitrile/water extractions resulted in all

good for all samples ranging from 84.9%-100.0%. For the phenyl labeled samples, the Methanol #1 Eluates were further analyzed by 2D-TLC and HPLC. The Methanol #1 Eluates from the triazine labeled samples were characterized by 2D-TLC, but there was not sufficient ^{14}C present for HPLC analysis, (Figures 8 and 9).

The Water Eluate from the phenyl labeled day 28 nonedible aqueous fraction was characterized by 2D-TLC and HPLC (Figure 5). There was not sufficient ^{14}C-material for analysis of the Water Eluate from the triazine labeled day 14 nonedible aqueous fraction, Figure 9.

The aqueous fraction of the day 28 phenyl labeled edible tissue was also purified on a C18 Bond Elut (Figure 3). Due to the extremely low levels of radioactivity found in the tissue, quantitative recoveries could not be determined. The Methanol #1 eluate and the Water Eluate were subjected to 2D-TLC analysis.

There was not sufficient ^{14}C present in the other aqueous fractions of the edible tissues for further analysis.

Thin Layer Chromatography

The organic fractions from the nonedible tissues were chromatographed directly by 2D-TLC. The autoradiograms for days 14 and 28 from the phenyl labeled tissues are shown in Figures 11A and 11B, respectively. There was one ^{14}C region present, zone 1, which cochromatographed with parent, CGA-131036. Figure 11C and 11D show the autoradiograms from the triazine labeled organic fractions. Again, there was only one component, zone 1 or CGA-131036.

Two-dimensional TLC was also used to analyze the partially purified organic fractions of the edible tissues (Methanol Eluates from Silica SepPaks). Figures 12A and 12B show the 2D-TLC autoradiograms obtained from 14 day and 28 day samples from the phenyl label. Zone 1 was the only component detected. The 2D-TLC profile of the triazine labeled organic fraction of the 14 day edible tissue is shown in Figure 12C. Zone 1 or parent was the predominant component. A minor, unknown metabolite, zone 5, was found only in the triazine label. A trace of zone 2 was detected. Zone 2 cochromatographed with CGA-150829. Analysis of the organic fraction of the 28 day edible tissue sample was not successful due to extremely low levels of ^{14}C.

High Pressure Liquid Chromatography

The organic fractions of the nonedible tissue were further characterized by HPLC. Figures 15A and 15B show the ^{14}C histogram obtained from phenyl labeled samples exposed for 14 and 28 days, respectively. There was one predominant component, Peak 1. This peak had the same retention time as CGA-131036. A small amount of polar material eluted in the solvent front for the 14 day tissue. The HPLC profile of the triazine labeled organic fractions was similar to the phenyl labeled samples, Figure 16A and 16B. Peak 1 was the major component; low levels of the polar peaks 2 and 3 were found.

To confirm that Peak 1 was parent, cochromatography of triazine-^{14}C-CGA-131036 with the 14 day samples from each label was performed. As Figure 17 shows, Peak 1 cochromatographed with triazine-^{14}C-CGA-131036 for both samples, thus confirming the TLC results.

There was not sufficient radioactivity in the organic fractions of the edible tissue for HPLC characterization.

The aqueous fractions after C18 Bond Elut purification were also characterized by HPLC. Only the phenyl labeled samples were present in sufficient quantities for analysis. The HPLC profile of the Methanol #1 Eluate from the 14 day sample is shown in Figure 18. The major component was Peak 1 or parent.

Figures 19A and 19B contain the HPLC profiles of the Methanol #1 Eluate and the Water Eluate from the aqueous fraction of the 28 day phenyl labeled sample. Some parent was present, Peak 1. Other polar components were also found in Peaks 2 and 3. Very low levels of these metabolites made further analysis impossible.

These HPLC data confirm the 2D-TLC data indicating that parent was the major component in the nonedible tissues, regardless of the time. Peaks 2 and 3 contain polar metabolites, possibly conjugates of the phenyl ring.

Quantitation of Metabolites

The organic and aqueous fractions of the nonedible tissues were quantitated by 2D-TLC, Tables VII and VIII. Regardless of the label, parent was the major

limit of detection to 0.33 ppm. Levels in the nonedible tissues were slightly higher, 2.55 ppm to 6.46 ppm. Two dimensional-TLC and HPLC were used to characterize metabolites in the edible and nonedible tissues whenever sufficient ^{14}C was present for analysis.

These results showed that metabolism of CGA-131036 in bluegill sunfish was not extensive. Parent was shown to be the major component in the nonedible tissues at 14 and 28 days for both labels. Cleavage of the sulfonyl urea bridge was minimal indicating this was a minor metabolic pathway. Two-dimensional TLC results suggested the presence of CGA-198638. Hydroxylation of the phenyl ring to form polar conjugates was a possible route of metabolism, Figure 20. Because of the extremely low levels of radioactivity present, mass spectral analysis of these components was not possible.