levels of DDT exceeded ambient water concentrations by 40-50 fold. It seems likely that uptake was largely via the gills and that transfer from gills to plasma occurred readily. Even allowing for trapped blood, the DDT plus metabolite level of most tissues exceeded that of ambient water.

Because of the high level of total radioactivity in liver (about 30 ppm) and because liver is a likely site for metabolism of DDT, we analyzed acetonitrile extracts of liver from present eels using thin layer chromatography as described elsewhere in this Bulletin (Pritchard, Guarino, and Kinter). There were no significant differences between livers from sea- and fresh-water adapted animals and most of the radioactivity (average 82%) was associated with the parent compound, DDT. About 10% of the radioactivity occurred as DDD, while DDE and DDA each accounted for about 4% of the total counts. These results suggest that DDT is metabolized at a moderate rate in the eel (on the order of 18% in 6 hr.) and that the tissue levels reported in Table 1 reflect primarily the parent DDT. In addition, the contrast between 18% metabolites in eel liver and only 5% metabolites in winter flounder liver under equivalent experimental conditions (Pritchard, Guarino, and Kinter) points to a marked species difference between these two teleost fish. Evidently, DDT metabolism and excretion will have to be evaluated fully in each species investigated.

Returning to the distribution data in Table 1, the brain level of about 10 ppm is interesting in light of the hyperactivity and convulsions observed in all eels after 4-6 hr. of exposure to 1 ppm DDT. Moreover, the gill and gut mucosa levels of about 20 and 6 ppm, respectively, provide support for the view, expounded elsewhere in this Bulletin (Janicki and Kinter), that the eventual death of eels exposed to 1 ppm DDT (after 8-10 hr.) results in part from specific impairment of osmoregulation. Under *in vitro* conditions such levels are sufficient to inhibit significant fractions of both the Na^+, K^+, Mg^{2+}-ATPases and the active Na^+ transport in these two osmoregulatory organs.

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1971 #11

RENAL AND HEPATIC EXCRETION OF PHENOL RED AND ITS GLUCURONIDE IN THE DOGFISH, *Squalus acanthias*.

A.M. Guarino, J.B. Call, H.A. Bering, and R.H. Adamson, Laboratory of Chemical Pharmacology, National Cancer Institute, Bethesda, Md.

In 1912 Rowntree and Geraghty (Arch. Int. Med. 9, 284,) introduced phenolsulfonephthalein (PSP, phenol red) as a renal function test. In most mammals the majority of the injected dye is excreted by the renal tubules while some is excreted by the liver into the bile. Although phenol red is metabolized to a glucuronide by the rat (Hart and Schanker, Proc. Soc. Exp. Biol. Med. 123, 433, 1966), the usual clinical assay, which is also used extensively in other research, measures only the free drug. Since we had shown (Adamson and Guarino, Comp. Biochem. Physiol., in press, 1971) that the dogfish also excretes phenol red glucuronide in its bile, it was of interest to compare the hepatic and renal excretions of this compound.

Female dogfish weighing from 3-6 kg were prepared for collection of urine by ligating one end of a 10 cm length of PE 190 tubing to the urinary papillae and the free end to a rubber balloon.

Injections of phenol red (10 mg/kg) were given via the caudal vein, and blood samples were drawn at specified time intervals. After sacrificing the animals, urine, gall bladder bile, kidney, and liver samples were collected and measured for total volume or weight. Assays were conducted essentially as described by Hart and Schanker, 1966, and the results are shown in Table 1. It can be seen in this

Table 1

RENAL AND HEPATIC HANDLING OF PHENOL RED AND ITS GLUCURONIDE IN THE DOGFISH*

	10 min	1 hr	2 hr	4 hr	24 hr	48 hr
Plasma, Free	63 ± 8	39 ± 20	20 ± 12	15 ± 4	<5	<5
Kidney, Free	222 ± 63	-----	183 ± 56	9 ± 6	5 ± 1	<5
Urine, Free	<5	<5	890 ± 259	711 ± 430	238 ± 139	-----
Urine, Glucuronide	<5	<5	184 ± 34	63 ± 34	58 ± 27	-----
Liver, Free	15.8 ± 4.4	-----	58.7 ± 17.2	37.8 ± 9.9	<5	<5
Bile, Free	<5	<5	250 ± 128	262 ± 125	2643 ± 936	3963 ± 1680
Bile, Glucuronide	<5	<5	79.4 ± 40.4	120 ± 56	882 ± 690	1532 ± 1245

*μg/ml or g ± SD

table that the drug appears more rapidly in the kidney than it does in the liver; 2 hr. after injection the kidney/plasma ratio was 9.2 while that of liver/plasma was 2.9. Consideration of the urine/plasma (U/P) ratios gives the expected high values of 47 and above. Between 2 and 24 hrs. after administration of phenol red 8.5 - 22.8% of the urinary excretion material occurred as the glucuronide. The Bile/Plasma (B/P) ratios were even more elevated (>753) and up to about 4 mg/ml of the free drug appeared in the bile while about 1.5 mg/ml occurred in bile as the glucuronide after 48 hrs. Between 2 and 48 hrs. the glucuronide represented 25-31% of the excreted material appearing in bile.

When these data are presented in terms of percentage of administered dose in a given tissue or body fluid, Table 2, one can compare the relative importance of the renal and hepatic compartments. It can be seen that the plasma levels decay rather rapidly with a $t_{1/2}$ of about 1 hr. while the 24 hr. levels are below the limits of the assay. The amount of free phenol red reaching the urine and the bile in 24 hrs. is about the same, or equal to about 25% of the administered dose. Phenol red glucuronide begins to appear in both urine and bile by 2 hrs. with more of the total dose appearing in the urine (12.2%) than in the bile (3.2%) at this time interval. This trend continues at 4 hrs. but by 24 hrs. the biliary route has excreted slightly more of the dose than did the renal route (38% *vs.* 31%). In studies where bile was sampled 48 hr. after injection nearly one-half of the dose was excreted via this route. Hence, the dogfish appears to provide an excellent animal for the type of pharmacokinetic modeling which seeks to describe the dominance of particular excretion routes. A. Despopoulos (Am. J. Physiol. 210, 760, 1966; and *Ibid* 220, 1755, 1971) has postulated a congruence of renal and hepatic excretory functions for organic anions and has had to resort to a variety of techniques,

Table 2

COMPARTMENTAL ANALYSIS OF PHENOL RED AND ITS GLUCURONIDE IN THE DOGFISH*

	10 min	1 hr	2 hr	4 hr	24 hr	48 hr
Plasma, Free	25.4 ± 3.0	15.4 ± 8.1	7.8 ± 4.6	6.1 ± 1.7	<2	<2
Kidney, Free	4.0 ± 1.5	-----	1.7 ± 0.8	0.2 ± 0.1	0.1 ± 0.1	<.01
Urine, Free	<1	<1	9.4 ± 4.1	13.3 ± 7.6	23.8 ± 11.8	-----
Urine, Total	<1	<1	12.2 ± 4.6	14.7 ± 8.3	30.6 ± 12.8	-----
Liver, Free	17.8 ± 4.7	-----	53.3 ± 9.5	41.5 ± 2.1	<5	<5
Bile, Free	<1	<1	2.4 ± 1.5	2.6 ± 1.6	28.0 ± 13.7	35.7 ± 16.0
Bile, Total	<1	<1	3.2 ± 2.0	3.7 ± 2.1	38.1 ± 19.2	49.5 ± 11.5

* % Dose in indicated compartment

intact animal, organ perfusion, and tissue slice in attempts to support his hypothesis. As far as phenol red is concerned the dogfish appears to be an excellent model because by the use of an intact animal, we have been able to show that this drug is handled about equally by renal and hepatic mechanisms.

1971 #12

THE FATE OF RADIOCARBON METHYL MERCURY IN THE LOBSTER

A.M. Guarino, J.B. Call, J.B. Pritchard, P.D. Briley and D.P. Rall, Laboratory of Chemical Pharmacology, National Cancer Institute, Bethesda, Md. and National Institute of Environmental Health Sciences, Research Triangle Park, N.C.

Because of the reported persistence of ^{14}C-DDT in the hepatopancreas of the lobster, it was of interest to investigate whether another important environmental pollutant, methyl mercury, behaved similarly after intravascular injection. Other studies were conducted to measure ^{14}C-methyl mercury tissue levels in lobsters after feeding DDT-contaminated food or after exposure to DDT-containing water. Lobsters weighing about 400-500 g were procured locally (Lunts and Small Lobster Bar, Trenton, Maine). One group of animals received 1.0 mg/kg of ^{14}C-methyl mercury chloride (Amersham/Searle) (Me-Hg) in 50% ethanol by injection into the pericardial sinus. After administration of this material, animals were placed in a standard lobster crate, were submerged in Laboratory Cove at about 30 feet of water and were fed pieces of mackerel two times a week. After the time intervals indicated in Table 1, the animals were dissected, and further handling was as described elsewhere in this Bulletin (Janicki, Guarino, and Kinter, 1972). The distribution at 24 hrs is some-