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Fate of 7,12-Dimethylbenz(a)anthracene in Rainbow Trout, *Salmo gairdneri*

Ann R. Schnitz, Katherine S. Squibb, and Joseph M. O'Connor

Laboratory for Environmental Studies, New York University Medical Center, Tuxedo Park, New York 10987

Polycyclic aromatic hydrocarbons (PAH) are contaminants of surface waters and sediments, especially near urban centers (Malins et al. 1985). Although aquatic biota accumulate PAHs from environmental sources, metabolism may be rapid, and biota sampled from contaminated areas often have concentrations lower than might be estimated from bioconcentration factors. In some cases (e.g. benzo(a)pyrene; BaP) PAH metabolism by aquatic biota may create reactive intermediates, some of which have been related to chronic effects in fishes (Varanasi et al. 1981; Malins et al. 1985). This report describes the fate and distribution of 7,12-dimethylbenz(a)anthracene (DMBA) after oral administration to rainbow trout (Salmo gairdneri). Emphasis has been placed on the disposition of DMBA among tissues and on DMBA transformation in the hepatobiliary system.

MATERIALS AND METHODS

Sexually immature rainbow trout (41 ± 8 g; mean ± s) were obtained from Musky Trout Hatchery, Asbury, NJ. The fish were maintained in 300 l aquaria at 10.0 ± 0.4 C and treated with 60 ml MarOxy fungicide (Mardel Laboratories, Glendale, IL). Fish were held in the laboratory for at least 48 hr before use and were not fed during the experiments. Stock ^{14}C-DMBA (Amersham) had a specific activity of 32.34 uCi ^{14}C per mg. Dosing experiments used DMBA in acetone, added to corn oil; residual acetone was removed by a stream of N_2, and individual doses (655 ± 8 ng; 0.02 uCi per dose) were loaded into #5 clear plastic capsules (Eli Lilly, Indianapolis, IN). Individual fish were dosed by gavage while under light anesthesia (MS-222; 3-aminobenzoic acid ethylester; Sigma), placed in clean water for recovery from anesthesia (≤ 10 min) and then moved to 105 l, all-glass holding aquaria.

Samples of four fish were removed from the holding tanks at 6, 12, 24, 48 and 72 hr after dosing. The fish were

killed with an overdose of MS-222 and muscle, gill, gall bladder, liver, stomach (including esophagus), intestine and trunk kidney were taken for analysis. Muscle, gill, stomach, intestine and trunk kidney were analyzed to determine total DMBA-derived ^{14}C radioactivity; no differentiation was made between ^{14}C activity derived from parent DMBA and DMBA metabolites. Tissue samples were digested in Protosol (New England Nuclear) at 55 C and fluored with Ecoscint (National Diagnostics) for counting on a Packard Tri-Carb Liquid Scintillation Counter (LSC).

Gall bladder and liver samples were fractionated to separate parent DMBA from more polar, DMBA-derived metabolites. Samples were homogenized (2X) in cold methanol/chloroform (MeOH/CLF, 1:1 v/v). After centrifugation the solvents were decanted and the pellet saved. Excess water was added to the MeOH/CLF mixture, the samples were centrifuged, and the two solvent layers were separated for analysis by LSC. Aliquots of the CLF and MeOH/water phases were reduced in volume, fluored, and the samples counted. Pellets were digested in Protosol, fluored and counted to determine solvent extraction efficiency (approximately 80%).

RESULTS AND DISCUSSION

Less than 0.06% of the administered ^{14}C activity was detected in trunk kidney samples from any sampling period; data from other tissues are presented here.

Whole-body retention of an oral dose of DMBA in rainbow trout was relatively inefficient; no more than 12.4% of the administered dose was found in non-alimentary tissues at any time (Table 1). DMBA-derived radioactivity in the whole body, including the gut, declined from 100% to 53% of the administered dose by 6 hr after dosing, followed by a decrease to 16% of the administered dose between 6 and 72 hr after dosing. Applying first-order kinetics to the data, the calculated rate-constant for whole-body elimination of DMBA-derived activity from the rainbow trout (k_e) was 0.02 hr^{-1}, yielding an elimination half-time for the ingested dose of 34 hr. Almost 30% of the administered dose remained in the stomach and intestine 48 hr after dosing (Table 1).

Accumulation of DMBA-derived radioactivity in non-alimentary tissues of rainbow trout was rapid during the first 12 hr after dosing. Accumulation thereafter was slow, with the maximium burden (80.6 ng; 12% of the administered dose) occurring 48 hr after dosing (Table 1). Most of the DMBA in the trout (1.66% of the administered dose) was in the muscle and gill 6 hr after

Table 1. Percent of the original DMBA dose remaining in tissues of rainbow trout following administration of a 655 ng dose. Data for tissues and whole body given as percent dose (± 1 standard error of the mean; n = 4). Total non-alimentary data are the sums of mean values from muscle, gill, gall bladder and liver.

Time after Dosing	Tissue							
	Muscle	Gill	Gall Bladder	Liver	Total Non-Alimentary	Stomach	Intestine	Total
6	0.74 (0.33)	0.92 (0.66)	0.30 (0.04)	0.34 (0.10)	2.30	45.71 (3.18)	4.77 (2.42)	53.08 (3.26)
12	2.76 (0.74)	0.50 (0.21)	2.24 (0.79)	0.71 (0.03)	6.21	33.45 (4.07)	32.61 (9.76)	67.91 (18.75)
24	1.84 (0.40)	0.25 (0.03)	3.33 (0.33)	0.87 (0.20)	6.29	19.27 (10.65)	8.87 (3.96)	32.56 (14.38)
48	2.05 (0.30)	0.50 (0.01)	9.06 (0.37)	0.69 (0.10)	12.30	13.90 (3.45)	14.58 (3.23)	40.84 (4.80)
72	1.63 (0.48)	0.14 (0.01)	6.46 (1.57)	0.74 (0.20)	8.97	4.66 (1.31)	2.92 (0.84)	16.24 (3.09)

Table 2. Mass of DMBA retained (ng) and proportional distribution (percent of retained dose) among non-alimentary tissues of rainbow trout between 6 and 72 hr after oral administration of a 655 ng dose of 14C-DMBA.

Time after Dosing	ng DMBA retained	Percent of Retained Dose			
		Muscle	Gill	Gall Bladder	Liver
6	15.1	32.2	40.0	13.0	14.8
12	40.6	44.5	8.1	36.1	11.4
24	41.3	29.2	4.0	52.9	13.8
48	80.6	16.7	4.1	73.6	5.6
72	58.0	18.2	1.6	72.0	8.2

dosing (Table 1). However, the DMBA burden in the liver and gall bladder increased rapidly. When expressed as a percentage of the DMBA present in the non-alimentary tract tissues at each sampling time, the DMBA-derived radioactivity in the combined liver and gall bladder samples increased from 27% of the retained dose 6 hr after dosing to 80.2% 48 hr after dosing (Table 2).

DMBA activity in the liver was transformed rapidly from non-polar, CLF-extractable material to less non-polar material extracted into the MeOH/water fraction. The total mass of DMBA-derived material in the liver was small, ranging from 2.2 ng 6 hr after dosing to 5.7 ng 24 hr after dosing (Table 3). Distribution of DMBA-derived activity between CLF- and MeOH/water-extractable material in the liver changed dramatically throughout the experiment, from approximately equal distribution after 6 hr, to the condition where 98% of the DMBA-derived ^{14}C-activity in the liver was in the form of MeOH/water extractable material 72 hr after exposure (Table 3).

Analysis of samples from gall bladder showed that both non-polar and less non-polar materials were transported rapidly from the liver to the gall bladder. Six hr after dosing the gall bladder had accumulated 13% (about 2 ng) of the DMBA-derived material present in non-alimentary tract tissues (Table 2). This value increased rapidly until 48 hr after dosing, when the gall bladder contained 73.6% of the retained dose, or about 59 ng of DMBA-derived material (Table 2). DMBA-derived activity in the gall bladder was present primarily as MeOH/water-extractable material, which comprised between 80% to 93%

Table 3. Mass of DMBA-derived material (ng) in liver and gall bladder and percent distribution of chloroform- and methanol-extractable DMBA-derived radioactivity in liver and gall bladder of rainbow trout exposed to an oral dose of 655 ng DMBA in corn oil.

	Liver			Gall Bladder		
Time after Dosing	ng DMBA activity	% in $CHCl_3$	% in MeOH	ng DMBA activity	% in $CHCl_3$	% in MeOH
6	2.2	44	56	2.0	20	80
12	4.6	39	61	14.7	13	87
24	5.7	16	84	21.8	9	91
48	4.5	22	78	59.3	9	91
72	4.7	2	98	41.8	7	93

of the DMBA-derived radioactivity in the gall bladder (Table 3).

A wide variety of PAH compounds have been detected in contaminated ecosystems, ranging from 2-ring compounds such as naphthalene to multi-ring PAH and heterocyclic compounds, including benz(a)anthracene and methyl-substituted benz(a)anthracenes (Bieri et al. 1986; Malins et al. 1985). Schultz and Schultz (1982) have studied DMBA-induced liver neoplasia in two species of Poeciliopsis, and Protic-Sabljik (1984) determined that DMBA induced AHH activity in carp, leading to the formation of mutagenic metabolites. We know of no other studies providing data on the assimilation and metabolism of DMBA in fishes.

The overall retention and tissue disposition of DMBA in trout can be compared to that observed for phenanthrene (3-ring PAH) and BaP (5-ring PAH) administered to other aquatic biota, including blue crabs (Callinectes sapidus), English sole (Parophrys vetulus) and pollack (Pollachius virens). Assimilation of DMBA and phenanthrene from the gut appears to be slow in fishes. Almost 30% of the administered dose of DMBA remained in the alimentary tract of trout for 48 hr in the present study, while Solbakken et al. (1980) reported that pollack dosed with phenanthrene retained as much as 47% of the administered dose in the intestine 48 hr after dosing. In blue crabs, however, Moese (1987) reported rapid removal of phenanthrene from the gut, with less than 1% of the administered dose remaining after 24 hr.

The elimination rate constant of phenanthrene in blue crabs was 0.07 hr^{-1}, more than a factor of 3 greater than estimated for DMBA in rainbow trout. Such disparate results may be due to the mode of administration of the dose; trout and pollack were dosed by placement in the gut of capsules containing PAH in vegetable oil, whereas Moese (1987) administered phenanthrene to blue crabs in a slurry made from blue mussel (Mytilus edulis).

Transport and tissue disposition of DMBA, phenanthrene and BaP appear to be similar among fishes. Solbakken et al. (1980) reported that about 13% of the phenanthrene dose administered to pollack was present in the bile between 36 and 96 hr after dosing. Similar results were obtained for DMBA; about 9% of the administered dose, comprising 73.6% of the non-alimentary DMBA burden, was present in the gall bladder 48 hr after dosing (Tables 1 and 2). Stein et al. (1984) exposed English sole to BaP-contaminated sediments and found that more than 98% of the BaP-derived radioactivity in the fish was present in liver and bile after 10 days of exposure. Varanasi et al. (1981) showed that BaP accumulation in the liver of English sole represented a small fraction of the administered dose (0.2 to 0.8%) between 16 and 336 hr after dosing. For trout, the fraction of the administered dose of DMBA in the liver never exceeded 0.87% of the administered dose (Table 1), and declined between 24 and 72 hr after dosing.

Like phenanthrene and BaP, DMBA transformation in liver tissue of fishes proceeds rapidly. In the present study between 78 and 98% of the DMBA burden in liver tissue was in the form of MeOH-extractable material from 24 to 72 hr after administration of a single dose of DMBA. During the same time between 91 and 93% of the DMBA-derived radioactivity in the gall bladder was present in the MeOH fraction. Varanasi et al. (1981) found that water-soluble metabolites of BaP comprised almost 98% of the BaP-derived radioactivity in liver tissue of English sole 24 hr after dosing; subsequent studies by Stein et al. (1984) determined that many of the BaP metabolites in English sole liver and bile are glucuronide conjugates. Solbakken et al. (1980) showed that pollack excreted phenanthrene in bile and in urine as either parent compound or as glucuronide and sulfate conjugates of hydroxy- and dihydrodiol-phenanthrene derivatives. Studies are currently underway in this laboratory to determine the extent and nature of glucuronide and sulfate conjugation of DMBA metabolites in rainbow trout.

Like other multi-ring PAH compounds, DMBA was assimilated from the gut of rainbow trout and transported to tissues rapidly, with the majority of the body burden

retained in, or transported to, the hepatobiliary system. These results are consistent with the concept that liver metabolism, transport of parent compound and conjugated metabolites to the gall bladder, and excretion to the intestine, is the primary route for PAH elimination in fishes (Solbakken et al. 1980; Stein et al. 1984). The identification of the DMBA metabolites formed in trout liver has not been completed; however, studies of BaP metabolism with trout and other fishes suggest that reactive metabolites capable of binding to trout liver DNA may be produced (Gmur and Varanasi 1982).

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