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DETOXIFICATION OF METALS AND ORGANIC COMPOUNDS IN WHITE CROAKERS

Greater than normal concentrations of contaminants are commonly found in the tissues of marine animals living in contaminated conditions. Even though in some instances no changes can be seen under microscopic examination, the suspicion remains that some damage may exist. Until recently, there were no scientific tools available to determine whether specific contaminants were causing adverse effects at a subcellular level. The objective of research described in this and accompanying papers is to develop those tools and to show what happens to contaminants inside organisms.

The organisms tested to date have some capacity to keep contaminants away from sensitive cellular sites where toxic action could take place. Animals with increased levels of inorganic metals synthesize a protein called metallothionein which binds and detoxifies those metals. Animals with increased levels of synthetic organic compounds, such as DDT or PCB, initially partition these into lipid pools, and then metabolize them with a mixed function oxygenase system. The metabolites so created are effectively detoxified by being bound to an intracellular tripeptide called glutathione, as shown in Figure 1.

Under some special circumstances, such as a very large and abrupt loading of metal ions in a laboratory tank, the loading capacity of the animal's metallothionein can be exceeded and metals can spill over into sensitive cellular sites where enzymes can be affected (Brown *et al.*, 1977; Brown and Parsons 1978). Similarly, acute exposures to synthetic organic compounds may overload lipid pools with these, and they may then spill over into sensitive sites including nerves (Quraishi 1977; Porter and Wiemeyer 1972; Young *et al.* 1979). However, at probable environmental levels of chronic exposure, this is not likely to occur. Rather, in chronic exposure, direct toxic effects of organic compounds will occur only if the level of metabolites exceeds the loading capacity of glutathione and other similar conjugating agents, and these metabolites spill over onto sites of toxic action including the enzyme pool (Brodie, *et al.* 1971; Gillette *et al.* 1974; and Shimada 1976). Thus the cellular enzyme pool is a site of toxic action for both metals and organic metabolites. Only those contaminants which can be shown to be present at a site of toxic action can be said to be causing direct toxic effects at that site.

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The data in this study show that all measured metals and organic metabolites are detoxified in croakers collected from near Dana Point, a relatively clean control site. However, in croakers collected from near the JWPCP outfall at Palos Verdes, trace organic metabolites appear to be present at levels which slightly exceed the capacity of their detoxification system. These excess metabolites appear in the metallothionein- and enzyme-containing pools and, therefore, may be responsible for reduced metal binding and enzyme activities measured in these pools. Surprisingly high concentrations of metabolites relative to parent compounds were found, indicating that both must be measured if one is to obtain a true assessment of synthetic organic contamination in the environment.

METHODS

We have devised a simple means for determining the partitioning of specific contaminants between sites of detoxification and sites of toxic action. This involves the separation of a tissue extract into three distinct pools: (ENZ) a high molecular weight (20,000 - 75,000 daltons) enzyme-containing pool which is a site of toxic action for both metals and organics; (MT) a medium molecular weight (3,000 - 20,000 daltons) metallothionein-containing pool which is a site of detoxification for trace metals; and (GSH) a low molecular weight (< 3,000) daltons) glutathione-containing pool which is a site of detoxification for trace organic metabolites. This separation is done by homogenizing the tissue, centrifuging it at $105,000 \times g$ for 60 min, collecting the supernatant, and passing it through a column packed with Sephadex G-75 gel. This final step separates the tissue extract according to molecular weight to produce the three distinct pools described above. A number of fractions (usually 35) are collected and trace metal

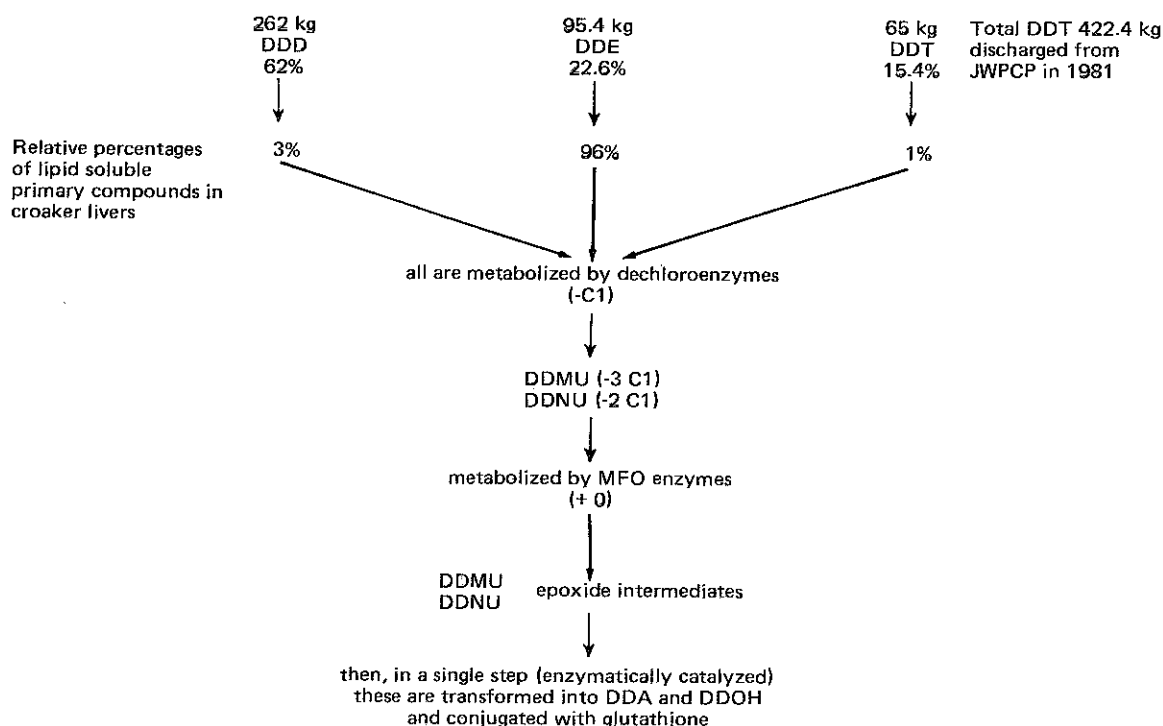


Figure 1. Schematic pathway of the metabolic processes in liver tissue that effectively detoxify the DDT, DDE and DDD (total DDT). MFO: mixed function oxygenases.

levels determined in each of these. Fractions comprising each of the three pools (identified by molecular weight markers and metal profiles) are then pooled and metabolites are extracted using a heat-catalyzed base hydrolysis to liberate conjugated metabolites (Gold *et al.*, 1981).

In the present study, croakers (*Genyonemus lineatus*) were collected by otter trawl at a depth of 60 m in September 1981 from two sites near Dana Point and Palos Verdes on the coast of southern California. Dana Point is considered to be a relatively clean site, but sediments near Palos Verdes are highly contaminated with trace metals and organic compounds (Word and Mearns, 1979). The lipid weight concentration of DDT was determined in liver and muscle tissues of the croaker since unmetabolized synthetic organics are not acutely toxic as long as they remain partitioned into lipid reservoirs (Porter and Wiemeyer 1972). Metals (Cd, Cu and Zn) and DDT metabolites (DDA and DDOH) were examined to determine their partitioning between sites of detoxification (MT and GSH) and a site of toxic action (ENZ). This latter analysis was done in liver tissue for DDT metabolites and in liver, kidney, intestinal, and muscle tissues for metals.

The activity of the enzyme lactic dehydrogenase was determined in the ENZ pool of liver extracts, as a marker of toxicity at the molecular level. Livers were also examined histopathologically to determine effects at a cellular level. Histopathological examination was done using a blind rating system so that the examiner did not know from which location the examined fish were taken. All conditions were rated from zero to three, where zero was best and three was worst. Statistical significance of results was analyzed using a Chi Square test.

TRACE METAL DETOXIFICATION/TOXIFICATION

In this section it will be shown that excesses of essential metals and nonessential metals are effectively detoxified since they are partitioned into the MT pool away from sensitive cellular sites such as enzymes. Sephadex G-75 gel elution profiles of composites of liver cytosols (soluble components) of ten croakers from Dana Point and ten from Palos Verdes are shown in Figure 2. Highest molecular weight molecules are eluted first and lowest molecular weight molecules last. Fractions 1-15 constitute the ENZ pool, fractions 16-28 the MT pool, and fractions 29-35 the GSH pool. The concentrations of metals in fractions comprising each of these three pools have been added and expressed as a wet weight concentration of metal in each of these pools for liver, kidney, intestines (cleaned), and muscle (Figure 3).

Copper and zinc were present in the ENZ pools of all tissues (Figure 3) because these are essential components of over one hundred metalloenzymes (Friedberg 1974; Underwood 1971). Essential trace metals in excess of the requirements of metalloenzymes in the ENZ pool are partitioned into the MT pool (Brown and Chatel 1978). These are toxic when present in the ENZ pool at levels higher than required (Friedberg 1974).

In croaker liver from Dana Point or Palos Verdes, there was approximately one-half the amount of Zn in the MT pool than was in the ENZ pool, but approximately 5-8 times more Cu in the MT pool relative to the ENZ pool (Figure 3). Thus, Zn was present in a small excess and Cu in a large excess, but excesses were not harmful since these were partitioned into the MT pool.

Cadmium, a nonessential metal, occurred almost exclusively (94.5 to 97.2%) in the MT pool of livers of croakers from Dana Point or Palos Verdes (Figure 3). Since this Cd was very effectively partitioned away from enzymes in the ENZ pool, it was rendered nontoxic and therefore could not be considered a threat to the liver enzymes.

Concentrations of Zn, Cu, and Cd were lower in liver profiles of croaker from the more contaminated Palos Verdes region than in those from Dana Point. Total tissue concentrations of these

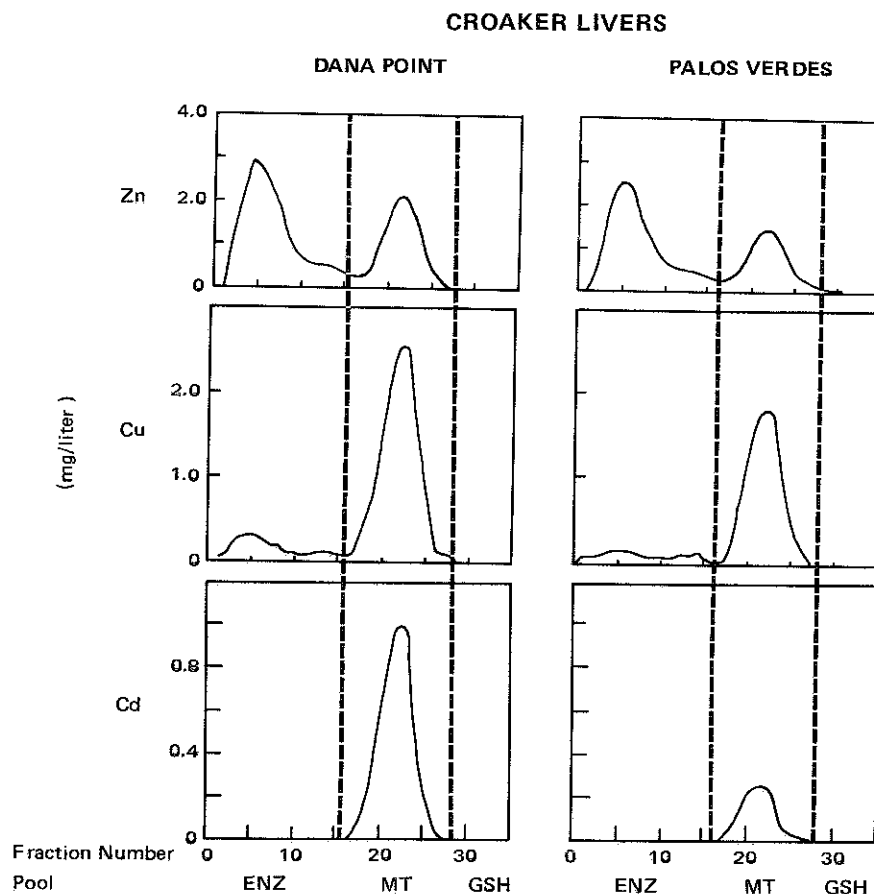


Figure 2. The concentrations (in mg/l) of Zn, Cu and Cd in individual fractions comprising the enzyme-containing (ENZ), the metallothionein-containing (MT), and the glutathione-containing (GSH) pools in Sephadex G-75 profiles of cytosol of croaker liver from lightly contaminated Dana Point and heavily contaminated Palos Verdes sites. In both cases Cu and Zn appear in the ENZ pool because they are essential components of metalloenzymes. Excesses of the essential metals, and the unessential metal, Cd are retained in the MT pool and prevented from reaching the ENZ pool where toxic effects could take place. Composites of 10 livers.

metals were also significantly lower in livers of Palos Verdes croaker (Table 1). However, when corrected for tissue dilution, due to the larger size of the livers from Palos Verdes, only total mass of Cu and Cd were lower in livers of Palos Verdes croakers (Table 1). These lower metal levels occurred in Palos Verdes croaker livers in spite of the fact that metal levels in the sediments near Palos Verdes are elevated one to two orders of magnitude over those at Dana Point (Word and Mearns 1979). It may be that these metals are less available for uptake from sediments near Palos Verdes. An alternative explanation, discussed later, is that the differences may result from the effects of chlorinated hydrocarbons on MT metal-binding, and resultant retention of metals in tissues of Palos Verdes croakers.

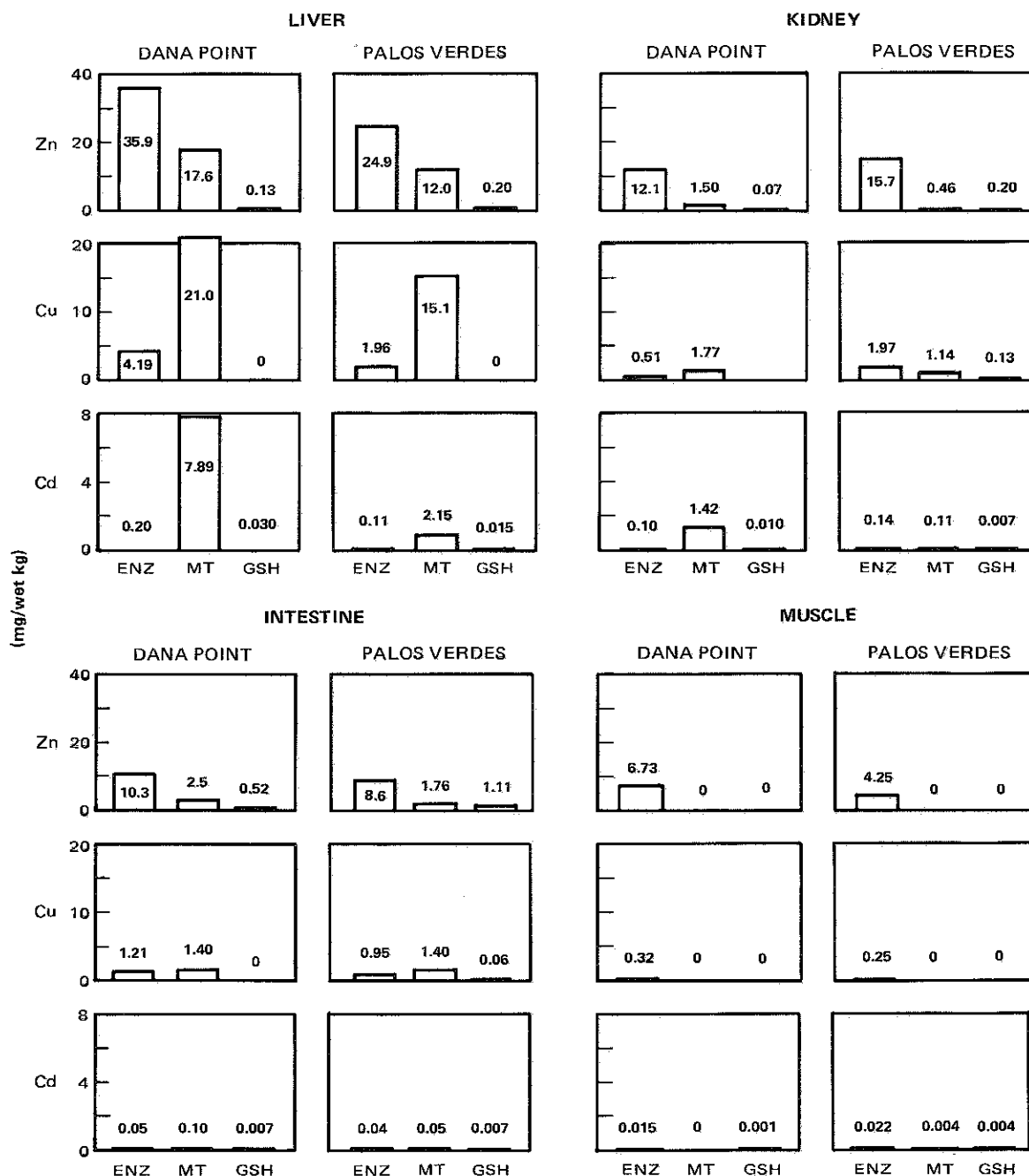


Figure 3. The concentrations of Zn, Cu, and Cd in the ENZ, MT and GSH pools expressed as mg/wet kg of tissue. These values were obtained by adding weights of metal in individual fractions comprising each of these pools (example profile in Figure 2) and dividing by the weight of tissue extract applied to the Sephadex G-75 column. Liver of croakers from Dana Point and Palos Verdes contained the highest concentrations of metals and had the greatest ability to detoxify metals as indicated by the highest levels of metals in the MT pool. Muscle had no metal detoxification capacity. Metal levels were lower in Palos Verdes profiles than in those from Dana Point, except for kidney Cu and Zn levels which were slightly elevated in the ENZ pools of Palos Verdes croakers.

Table 1. Concentrations of Zn, Cu and Cd were significantly lower in Palos Verdes croaker livers compared with those from Dana Point. However, when corrected for dilution due to the larger size of Palos Verdes livers, only masses (concentration x tissue wt) of Cu and Cd were lower. Muscle concentrations and masses were not significantly different.

	Liver Wt (g)	Zn	CONCENTRATION (mg/wet kg) Cu	Cd	Zn	MASS (ug) Cu	Cd
Dana Point (Control)	2.98	41.4 ± 4.7 ¹	24.9 ± 5.7	4.5 ± 1.4	123	74	13
Palos Verdes (Contaminated)	4.47	30.7 ± 2.1 ²	9.3 ± 3.0 ²	1.4 ± 0.7 ²	136	42	6
	Muscle Wt (g)						
Dana Point	142	3.46 ± 0.77	0.52 ± 0.12	0.020 ± 0.006	491	74	2.8
Palos Verdes	147	3.94 ± 0.71	0.45 ± 0.09	0.013 ± 0.005	579	66	1.9

¹ mean ± standard error; n = 10

² p < 0.05; 2-tailed t-test

In kidney profiles of Palos Verdes croakers, a higher portion of metals was in the ENZ pool than in the MT pool, when compared to those from Dana Point (Figure 3). There was four times as much Cu in the ENZ pool of kidneys from Palos Verdes croakers compared with those from Dana Point. This may indicate that kidneys of Palos Verdes croakers have a reduced ability to detoxify trace metals or that these metals are not retained in the kidney long enough to be detoxified.

There were slightly lower concentrations of metals in profiles of intestine of Palos Verdes croakers relative to those from Dana Point (Figure 3). This may indicate reduced uptake of metals in Palos Verdes croakers.

Concentration of Zn, Cu, and Cd tended to be lower in muscle profiles of Palos Verdes croakers relative to those from Dana Point (Figure 3). However, total tissue concentrations were not significantly reduced (Table 1). Only Cd appeared to show a trend towards lower levels on both a concentration and mass basis in muscle of Palos Verdes croakers relative to those from Dana Point.

Unlike the other tissues examined, virtually no metal was found in the region of the MT pool in muscle, indicating that this tissue has very little detoxification capacity (Figure 3). Most of the cytosolic Cu and Zn in muscle tissue was in the ENZ pool, most likely as essential components of metalloenzymes. Even though the concentrations of metals in muscle cytosolic pools were low relative to liver tissue, the total mass of metal in muscle was relatively high because of the large amount of muscle tissue (Table 1).

In summary, metal concentrations were generally lower in tissues of Palos Verdes croakers than those from Dana Point. In addition, a smaller portion of metals were bound to the MT pool in Palos Verdes croakers. These findings tend to support the hypothesis that croakers from Palos Verdes have a reduced ability to bind metals to MT resulting in reduced uptake, detoxification and retention of metals, including those which are essential for normal health.

SYNTHETIC ORGANIC DETOXIFICATION/TOXIFICATION

Acute effects of organic compounds can result from the release of parent compounds from lipid pools and their subsequent occurrence at sensitive sites including the lipid-containing membranes of nerve tissue (Porter and Wiemeyer 1972; Quraishi 1977). Chronic effects can result from the overloading of conjugating agents such as glutathione with metabolites, and their subsequent spill over onto macromolecules including enzymes (Gillette *et al.* 1974; McKinney 1981). In this section it will be shown that the lipid content of croaker tissues appears to be increased by exposure to synthetic organic compounds and that these appear to be partitioned into tissues according to their lipid content. It also will be shown that most organic metabolites are present in a nontoxic form in the GSH pool.

Croakers from Palos Verdes with higher concentrations of chlorinated hydrocarbons had higher liver weight, higher liver pellet weight, and higher liver and muscle lipid concentrations than those from Dana Point (Tables 2 and 3). The higher liver weight of Palos Verdes croakers resulted from both a higher lipid content and a higher pellet weight. The higher pellet weight is a reflection of an increase in endoplasmic reticulum (Allen *et al.* 1976). The endoplasmic reticulum functions in lipid synthesis and contains the enzymes that metabolize the parent trace organics compounds. The higher lipid content of liver and muscle tissue of croakers from Palos Verdes is in accordance with previous research showing increased lipid synthesis in response to chlorinated hydrocarbon exposure (Allen *et al.* 1976).

Apparently parent organic compounds were distributed among liver and muscle tissue according to lipid availability. The wet weight concentration of total DDT was higher in liver than in muscle of croakers from both Dana Point and Palos Verdes (Table 3). However, on a lipid weight basis, the concentrations of these parent organic compounds were nearly identical in liver and muscle tissue of croakers from either Dana Point or Palos Verdes. There was a larger total mass of lipid and parent chlorinated hydrocarbons in muscle tissue relative to liver tissue because there was so much more muscle tissue.

The concentrations of two DDT metabolites (DDA and DDOH) were measured in the ENZ pool, the MT pool, and the GSH pool in livers of all croakers (Figure 4). The levels of DDA and DDOH were 2 to 3 times higher in cytosolic pools of croakers from Palos Verdes than in those from Dana Point. Almost all of the metabolites in each of the three pools were conjugated to substances in each of these pools, because virtually none were extractable without a heat-catalyzed base hydrolysis (Gold *et al.* 1981).

Table 2. The amount of pellet and lipid recovered after centrifugation (105,000 x g) are increased in contaminated Palos Verdes croaker livers relative to those from the Dana Point control site. The increase of the pellet is probably a result of a proliferation of the endoplasmic reticulum, the cellular organelle responsible for increased lipid synthesis, and metabolism of organic compounds. Composites of 10 livers.

	Liver Wt. (g)	Pellet Wt. (g)	Lipid wt (g)	Water Soluble Fraction wt (g)
Dana Point	2.98	1.18 (39.6%)	0.06 (2.0%)	1.74 (58.4%)
Palos Verdes	4.47	2.29 (51.2%)	0.57 (12.8%)	1.61 (36.0%)

Table 3. The concentration and the mass (concentration x tissue wt) of chloroform/methanol extractable lipids are increased in Palos Verdes croaker liver and muscle as a result of higher tissue concentrations of Total DDT. The lipids sequester the lipophilic DDT's so that they are kept away from sensitive sites (e.g. nerves). The lipid wt concentrations of Total DDT are similar in liver and muscle tissue of croakers from the same location because chlorinated hydrocarbons are distributed among tissues according to lipid availability. Therefore the tissues with the highest mass of lipids have the highest mass of Total DDT. Composites of 10 tissues.

In Control area DDT: 6%, DDE: 94%, DDD: 0%
 In Contaminated area DDT: 1%, DDE: 96%, DDD: 3% Percentage of Total DDT's

	Liver Wt. (g)	Lipid Concentration (%)	Total DDT Wet Wt. Concentration (mg/kg)	Total DDT Lipid Wt. Concentration (mg/kg)	Lipid Mass (ug)	Total DDT Mass (ug)
Dana Point (Control)	2.98	6.0	0.55	9.2	0.17	1.6
Palos Verdes (Contaminated)	4.47	15.0	35.7	238	0.67	160
	Muscle Wt. (g)					
Dana Point	142	0.8	0.05	6.3	1.09	7.1
Palos Verdes	147	2.2	8.1	369	3.19	1191

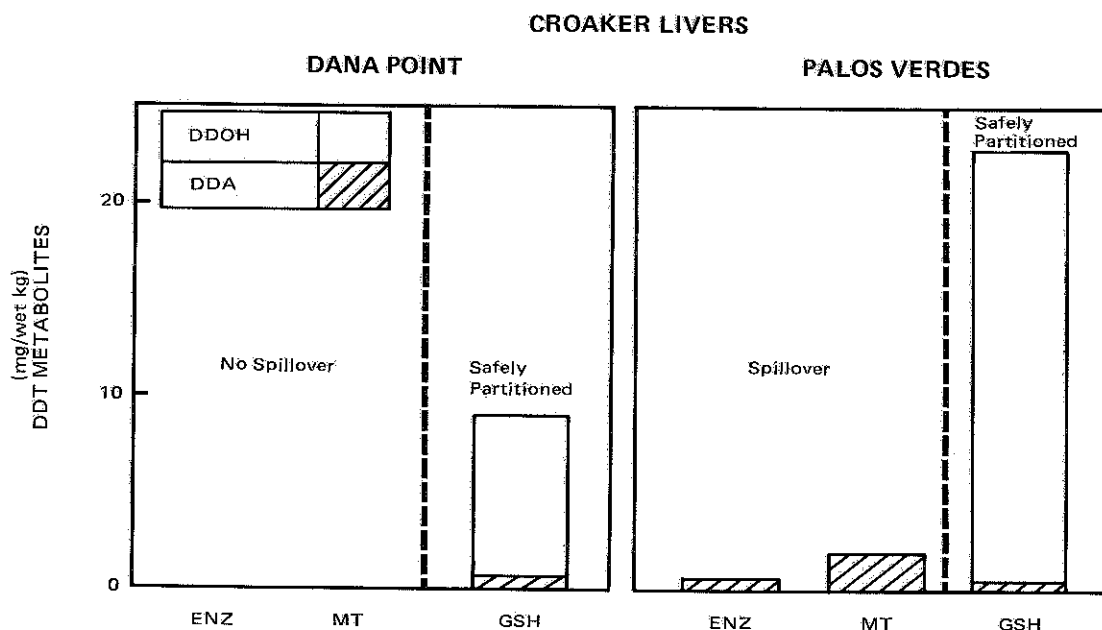


Figure 4. The distribution of the final metabolites of DDT, DDE, and DDD in the livers of croaker taken from Palos Verdes (PV) and Dana Point (DP) control. All the DDOH remains in the low molecular weight fraction for both sites where it is harmless. The DDA is at low levels in all three fractions of PV croaker liver indicating the possibility of toxic effects in composites of 10 livers.

In livers of croakers from Dana Point the metabolites measured (DDA and DDOH) were partitioned into the GSH pool. None occurred in the ENZ pool which is a site of toxic action. Thus, in livers of croakers from Dana Point, it appears that the loading capacity of the detoxifying GSH pool had not been surpassed. Since one of the major conjugating substances is glutathione in mammals (Brodie *et al.*, 1971; Shimada, 1976) and fish (James *et al.*, 1979), and since virtually all of the metabolites in the GSH pool were conjugated, we presume that they were, for the most part, conjugated to GSH. We are currently in the process of accurately determining the degree of saturation of GSH in this pool.

In livers of croakers from Palos Verdes all DDOH occurred as conjugates in the GSH pool and none occurred in the ENZ pool, indicating that DDOH was detoxified. However, not all DDA was detoxified in livers of croakers from Palos Verdes, since a large portion of it occurred in the ENZ pool. Most of the DDA that spilled over was found in the MT pool. Although it is known that metabolites conjugate with proteins once the loading capacity of GSH has been surpassed (Brodie *et al.* 1971; Shimada 1976), there have been no attempts to determine if metabolites conjugate specifically with MT.

In retrospect, our results are not surprising. Previous reports have indicated that metabolites attach to sulfhydryl groups in the amino acid cysteine, whether it is present in GSH or proteins (Miller and Miller 1966; Miller 1970). Both GSH and MT contain high proportions (33%) of their amino acids as cysteine while it constitutes a smaller portion of ENZ (7%). Therefore, once the loading capacity of sulfhydryls in GSH was saturated, metabolites would react more readily with the MT pool than the ENZ pool.

Since DDA occurs in the MT pool in livers of croakers from Palos Verdes, one might expect that it would reduce metal binding to that pool. Indeed, as stated previously, it appears that metal uptake, detoxification, and retention are reduced in several tissues of croakers from Palos Verdes. Previous research has indicated that there appears to be an inverse relationship between levels of metals and organics in organisms from southern California coastal waters (Young and Jan 1979). Other work on organic carcinogens indicates that these can reduce levels of metals bound to the MT pool of liver tissue and concomitantly reduce levels of trace metals (Brown *et al.* 1980). The present study indicates that organic compounds have an effect on metal metabolism when organic metabolites attach to binding sites in the MT pool. Reductions of metals in MT can be considered to be an adverse effect since MT is used as a reservoir for essential trace metals and as a detoxification mechanism for nonessential trace metals (Brown and Chatel 1978). Thus, the detoxification system for metals appears to be a site of toxic action for organics.

Since DDA occurs in the ENZ pool in livers of Palos Verdes croakers, it may be responsible for reduction of enzyme activities (Table 4) because enzymes are a prime site of toxic action of organic metabolites (Miller and Miller 1966). Since metal levels were reduced in this pool, reductions of enzyme activities cannot be attributed to excesses of metals in the environment.

The high proportion of metabolites relative to parent compounds (Table 5) was unexpected since previous studies have shown that DDT and DDE do not induce enzymes necessary for their metabolism in some fish species (Addison *et al.* 1977). We have considered several possible explanations for this apparent disparity. First, there may be large species differences in ability of organisms to metabolize various compounds (Peterson *et al.* 1979). Secondly, other organics present in these organisms could have stimulated production of enzymes which could metabolize DDT. In a concurrent study (Gossett *et al.*, this report) a large number of other organic compounds were measured in livers of croakers from Palos Verdes. Several other studies have

Table 4. The activity of the enzyme lactic dehydrogenase (LDH) is decreased in the enzyme-containing (ENZ) pool of Palos Verdes livers relative to those from Dana Point control sites, whether calculated in a tissue concentration basis or a mass (concentration x tissue wt) basis to correct for dilution due to the larger size of Palos Verdes livers. IU: International Units. Composites of 10 ENZ pools.

	Concentration (IU/Kg)	Mass (IU)
Dana Point	12,137	36.2
Palos Verdes	6,814	30.5

Table 5. The metabolites of DDT's comprise a large portion of the total of DDT and its derivatives in croaker liver. Since metabolites can be responsible for chronic effects, determination of levels of these, and the degree of detoxification, is most important. Data is in mg/wet kg. Composites of 10 livers.

	Parent Compounds Total Tissue DDT+DDE+DDD	Metabolites Cytosolic DDA+DDOH	Total Parent Compounds + Metabolites
Dana Point	0.55 (5.4%)	9.7 (94.6%)	10.3
Palos Verdes	35.7 (58.6%)	25.2 (41.4%)	60.9

suggested that pre-exposure or concurrent exposure to one compound can increase metabolism of another (Hart and Fouts 1963); Turusov and Chemeris 1976). Thirdly, there is much evidence to suggest that metabolites are retained for much longer periods of time than parent compounds in marine organisms (Lee *et al.* 1976; Malins 1977; Sanborn and Malins 1977). Therefore, even if metabolism of DDT, DDE, and DDD is very slow, there may be a continual buildup of metabolites relative to parent compounds.

The high proportion of metabolites found in fish in this study shows that, if one is to obtain a true assessment of synthetic organic levels in environmentally-exposed organisms, one must look at the total level of both parent compounds and metabolites (Table 5). This is true because organic compounds, which are metabolized as quickly as they are taken up, will not be detected unless metabolite levels are measured.

In summary, we have provided evidence that croakers have the ability to detoxify DDT, DDE, and DDD by increasing synthesis of lipids and by metabolism of these organics. Furthermore, we have shown that one DDT metabolite (DDA) is present at the sites of toxic action in livers of fish from Palos Verdes. These occurrences coincide with reduction of enzyme activity and metallothionein metal-binding capacity. Therefore, we suggest that DDT metabolites may be the cause of these effects.

HISTOPATHOLOGY

Histopathological examinations of liver tissue revealed changes which were commensurate with increased lipid deposition in liver of croakers from Palos Verdes relative to those from Dana Point. These conditions are summarized in Table 6 and Figure 5.

Hepatocytes (liver cells) in croakers from Palos Verdes have a vacuolated appearance due to increased lipid accumulation. In addition, hepatocytes were as much as twice as large in some croakers from Palos Verdes (Figure 5). This larger cell size accounts for the increased liver weight of croakers from Palos Verdes (Table 1). The increase of cell size, as discussed previously, is probably a function of both increased amounts of endoplasmic reticulum and increased lipid deposition.

Table 6. Normal and histopathological ratings of liver and skeletal muscle in the white croaker (example of valuation sheet)

Hepatic cord structure: 0 = normal, + = minor, ++ = moderate, +++ = major disarray
 Fatty hepatocytic vacuolation: 0 = normal, + = minor, ++ = moderate, +++ = severe
 Fat deposition in adipose cells: 0 = normal, + = moderate, ++ = mild, +++ = none
 Sinusoidal congestion: 0 = none/normal, + = mild, ++ = moderate, +++ = major
 Sinusoidal compression: 0 = none/normal, + = mild, ++ = moderate, +++ = major
 Melanin-macrophage centers
 (M-MC's): 0 = none/normal, + = infrequent, ++ = common, +++ = very common
 Intracellular hepatocyte: muscle glycogen ratio:
 0 = equal/normal, + = slightly more, ++ = moderately more, +++ = much more

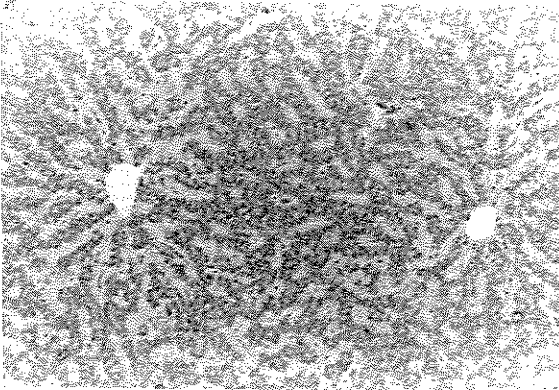
	DANA POINT ¹				PALOS VERDES ¹			
	0	+	++	+++	0	+	++	+++
Hepatic Cord Structure	2	8	0	0	2	6	0	2
Fatty Vacuolation of Hepatocytes	0	3	7	0	1	1	3	5***
Fat Deposition in Adipose Cells	3	1	3	3	2	0	2	6
Sinusoidal Pathology (Total)	6	6	2	6	1	6	5	8
Congestion	3	5	1	1	1	5	4	0**
Compression	3	1	1	5	0	1	1	8
M-MC Pathology (Total)	13	2	1	4	12	3	2	3
Vacuolation	9	0	0	1	4	3	1	2*
Hyperplasia/Hypertrophy	4	2	1	3	8	0	1	1
Parasite Incidence (Total)	11	2	2	6	11	0	2	7
Hepatic Protozoans	9	0	0	1	10	0	0	0
Intramuscular Protozoans	2	1	2	5	1	0	2	7
Intramuscular Helminthes	9	1	0	0	10	0	0	0
Lymphocytic Infiltrates	7	0	1	2	9	1	0	0
Degenerative Foci	8	0	0	2	10	0	0	0
Hepatopancreatic-Tubular Metaplasia	8	1	0	1	10	0	0	0
Intracellular Hepatocyte: Muscle Glycogen Ratio	9	0	0	1	4	0	1	5***

¹n = 10

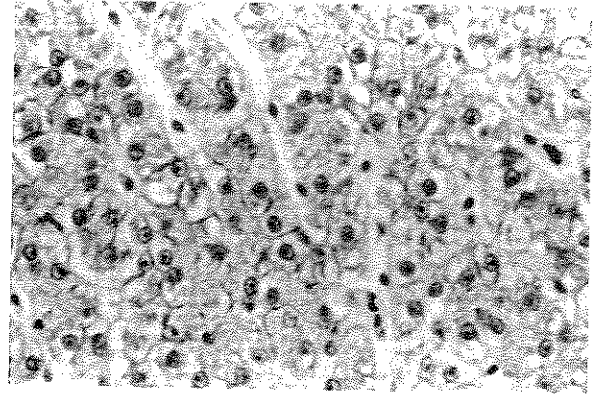
*p = 0.05

**p = 0.01

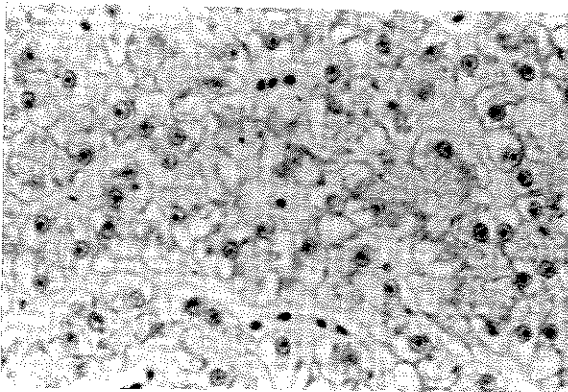
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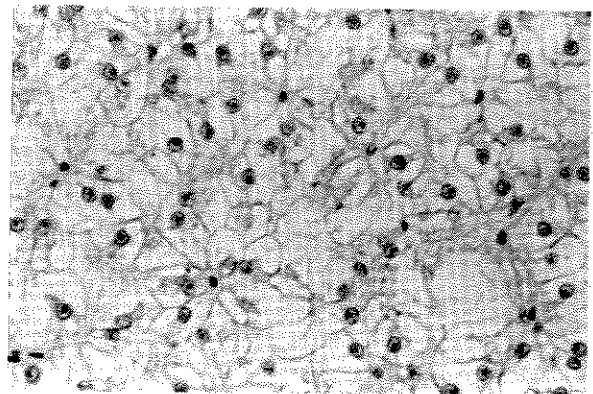
a. Liver parenchyma illustrating normal structure and radiating arrangement of hepatic cords and tubulosisinoids. Note the two central veins in their respective lobules; Palos Verdes. H. & E. X 125



b. Liver with mild fatty vacuolation of hepatocytes, occasional fat cells and tubulosisinoids, some of which contain nucleated red blood cells; Dana Point. H. & E. X 500



c. Liver with moderate fatty vacuolation of hepatocytes and congested tubulosisinoids, converging towards central vein (upper left); Dana Point. H. & E. X 250



d. Severe fatty vacuolation of hepatocytes. Compare with Figure 2, above; Palos Verdes. H. & E. X 500

Figure 5. Changes in liver tissue caused by lipid deposition concomitant with increasing chlorinated hydrocarbon tissue concentrations.

Sinusoids (the open spaces between cords of liver cells that radiate towards the central vein) were congested in livers of croakers from Palos Verdes relative to those from Dana Point. These function as a venous component of the circulatory system of the liver. In the livers from Palos Verdes, the hepatocytes were often swollen in size to a point where the sinusoids were completely closed off, impairing the flow of red blood cells.

It was also found that melanin-macrophage centers in the Palos Verdes livers had a vacuolated appearance, similar to the lipid-laden hepatocytes. These melanin-macrophage centers perform a phagocytic (waste collecting) function in the tissues of fish. The increased vacuolation of these may reflect increased lipid accumulation from the liver.

When livers were stained specifically for glycogen content, it was found that this was greatly increased in livers of croakers from Palos Verdes. Such an accumulation of glycogen in the liver is indicative of chronic stress, and most likely results from the stress-induced conversion of muscle glycogen to lactic acid, followed by its transport back to the liver where it is converted back into glycogen (Swallow and Fleming 1970; McLeay and Brown 1974).

In summary, histopathological examination indicates that croakers from Palos Verdes have larger hepatocytes, probably as a result of increases in endoplasmic reticulum and lipid production. These changes are probably a compensatory response to chlorinated hydrocarbons. However, they result in a blockage of the circulation of the liver and, therefore, may be harmful.

CONCLUSIONS

The synthetic organic compound DDT, its derivatives (especially DDE), and its metabolites (DDA and DDOH) were responsible for detrimental changes found in tissues of Palos Verdes croakers, including excess lipid accumulation in liver, reduced enzyme activities in liver, and reduced binding of metals by MT in several tissues. DDA appeared to have exceeded the loading capacity of the GSH pool in livers of Palos Verdes croakers. As a consequence, it spilled over into the enzyme- and metallothionein-containing pools. This spill over was most likely responsible for reductions of enzyme activity and metal level. As a consequence of a reduction of metal levels in the metallothionein-containing pool, tissue uptake and retention of trace metals may have been reduced.

Trace metals were not responsible for detrimental changes in tissues of Palos Verdes croakers since levels of these were lower than in croakers from the Dana Point control station. In addition, excesses of essential metals and nonessential metals were effectively detoxified by MT.

ACKNOWLEDGEMENTS

Portions of this study involving organic metabolites were supported by Grant No. NA80RAD-00040 from the National Oceanic and Atmospheric Administration (NOAA). A more detailed description of the research in this report is being published as three papers in *Pollution and Physiology of Marine Organisms - Fifth Symposium*, Academic Press, New York, Eds.: Vernberg, Vernberg, Calabrese and Thurberg. These are listed below in the reference section as papers by Brown *et al.* 1982, Jenkins *et al.* 1982 and Perkins *et al.* 1982.

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