TOXIC EFFECTS OF 2,3,7,8-TETRACHLORODIBENZO-*p*-DIOXIN (2,3,7,8-TCDD) AND RELATED COMPOUNDS (PCDD/PCDF) ON AQUATIC INVERTEBRATE SPECIES AND SPECIFIC STUDIES ON THE SOFT SHELL CLAM, (MYA ARENARIA)

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1. Introduction

Very few studies have examined the biological effects on aquatic invertebrate species following exposure to PCDD/PCDF type compounds.[1,2] The lack of interest in effects in this class of organisms has stemmed from the high nominal water concentration required to elicit acute toxicity (death in these organisms) when compared to higher vertebrates. Because of this apparent lack of response the concentrations resulting in sublethal toxicity were not evaluated. The lack of responsiveness was assumed to be due to the lack of an Ah receptor.[3] However, there was evidence of induction by polycyclic compounds, which would indicate that there was a receptor to initiate induction.[4] Brown et al.[5] have recently reported on a 28 and 39 kDa proteins that binds to the TCDD photoaffinity analogue and may be homologous to the Ah receptor in higher vertebrates. We are reporting on studies which examined sublethal lesions and tissue distribution in the Soft-shell clam (*Mya arenaria*). In addition, the biochemical and histological lesions reported in this paper will be compared to other invertebrates and effects seen in higher vertebrates.

2. Materials and Methods

Animals: The Soft-shell clams were purchased from Warren Denton Seafood (Prince Frederick, Md.) from Chesapeake Bay stocks previously shown to contain very low concentrations of PCDD/PCDF (0.3 pg/g, I-TEQ). There were very few histological lesions observed in this population of clams. They were maintained in 100 gallon glass aquaria until dosing, when they were transferred to smaller aquaria. The routes of 2,3,7,8-TCDD exposure was by gavage of the incurrent siphon into the pallial chamber, injection into the siphon, and water-borne exposure.

Chemicals: [^{3}H]2,3,7,8-TCDD (20.5 Ci/mM) was purchased from Chemsyn Science Laboratories (Lenexa, KA). 2,3,7,8-TCDD was purchased from Cambridge Isotope Laboratories (Woburn, Mass.) and the Calcium-45 was purchased from New England Nuclear (Boston, Mass.). All radioactive counting was carried out on a Tracor Mark III scintillation counter (Tracor Analytic, Elk Grove Village, IL) and values are reported on a DPM/gram wet weight of tissue after background subtraction.

Histology: Animals were fixed in 10% buffered formalin, dehydrated, embedded in paraffin and stained hematoxylin and eosin. The lesions were graded as to severity: absent, mild, moderate and severe. Lesions at the moderate and severe level would result in altered normal physiological function.

Experimental Protocols: In the first set of experiments the absorption and tissue distribution of 2,3,7,8-TCDD was evaluated in Mya following exposure by gavage, water-borne and injection on days 1, 3, 7, 14, 21, and 28. Because of the similarity in uptake by the three routes only the gavage is reported.

In the dose response study clams were dosed through the water with 2,3,7,8-TCDD dissolved in nonane. The clams were exposed for 24 h and then removed to clean water. The nominal concentrations tested were 2.0-2.5, 20-25, 200-250, and 2,500 ppt (ng/L) and there was a nonane control group. Ten clams from each group were sampled over a 4 week period and the total lesions calculated.

An experiment was carried out to evaluate the effect of a bolus gavage dose of 2,3,7,8-TCDD (200 ppt in nonane) on the total clam weight (TW) to the total shell weight (SW) ratio. Clams (N=10) were sacrificed at 1,3, 7, 14, 21, and 28 days post exposure.

Calcium-45 was used to evaluate calcium incorporation into the shell matrix of clams exposed for 24 h to 2,3,7,8-TCDD and then fourteen days later treated with Ca-45 and the calcium level determined 96 h later. The groups tested included a non-treatment control, a nonane control, and 2,3,7,8-TCDD at 2.5, 25, 250 and 2,500 ng/L.

3. Results and Discussion

The results reported below demonstrate that in bivalve mollusks that 2,3,7,8-TCDD can result in a number of similar effects as reported in higher vertebrates. The nominal concentrations that elicited these effects are generally higher than those for other species, but the target tissue dose would appear to be in the range for effects seen in several lower vertebrates. It is important to realize that the target tissue dose may be higher due to direct contact, lipid content and or some specific binding protein. The experiments described below used a nonane carrier for the 2,3,7,8-TCDD in order to increase the bioavailability to the clam and to simulate a lipid associated compound. The tissue levels (Table 1) achieved in these studies are in the range of environmental samples collected from contaminated areas.[6] However, the carrier solvent may have altered the toxicokinetics resulting in higher pulse doses than would be observed in the field since these compounds would be bound to particulate matter and less freely absorbed. The 2,3,7,8-TCDD was first associated with the gills, and then moved to the digestive tract. The

ultimate storage site for the 2,3,7,8-TCDD was the gonadal tissue (Figure 1, Table 1). The gonadal tissue is the site of 2,3,7,8-TCDD accumulation, which may be due to a tissue specific binding protein or the higher lipid content (2.47% wet weight basis).

TABLE 1. Absorption and distribution of [^{3}H] 2,3,7,8-TCDD in *Mya arenaria* following dosing by gavage at a dose of 2.0 pg/g on a total weight basis[A]

	DAY POST-EXPOSURE					
ORGAN	1	3	7	14	21	28
SIPHON	1.05 (0.34)	0.95 (0.47)	1.78 (0.33)	1.82 (0.34)	1.07 (0.28)	1.80 (0.89)
HRT/KID	2.68 (1.18)	2.55 (0.49)	3.88 (1.33)	4.69 (1.47)	3.40 (0.51)	4.87 (0.85)
GILL	6.59 (2.73)	4.76 (0.80)	3.39 (0.99)	2.67 (0.66)	2.24 (0.66)	2.92 (2.10)
MANTLE	1.39 (0.84)	1.63 (0.30)	2.18 (0.85)	1.87 (0.24)	1.74 (0.52)	3.43 (1.25)
SHL GLD[B]	1.87 (0.90)	1.56 (0.50)	2.06 (0.39)	1.81 (0.42)	1.30 (0.32)	2.23 (0.99)
DIG GLD[B]	4.90 (4.71)	3.83 (2.35)	5.32 (2.26)	4.80 (1.03)	4.30 (0.65)	6.27 (0.91)
MUSCLE	0.71 (0.45)	1.06 (0.46)	1.48 (0.50)	1.25 (0.29)	1.12 (0.34)	2.01 (0.80)
GONAD	0.69 (0.26)	2.15 (1.44)	6.27 (2.12)	9.46 (2.34)	8.49 (2.08)	8.93 (4.24)

A. Values in the table are the mean and (standard deviation) for pg ^{3}H-2,3,7,8-TCDD equivalents per gram wet tissue.
B. SHL GLD = Shell gland, DIG GLD = Digestive Gland.

One of the most consistent symptoms of this class of compounds is loss of body weight. The information in Table 2 demonstrates that the clams did decrease in body mass following dosing with 200 pg of 2,3,7,8-TCDD/gram of tissue (wet weight). The decrease in body weight was significant by day 14. Based on the toxicokinetic study the estimated target tissue dose for the gastrointestinal organs would be approximately 10-20 pg/g wet weight.

TABLE2. Alteration in total body weight to shell weight ratio following gavage dosing with a 200 ppt nominal dose[A]

	DAY POST DOSING					
	1	3	7	14	21	28
CLAM WT G/ SHELL WT G	1.330 (.145)	1.326 (.145)	1.168 (.092)	1.023* (.181)	1.017* (.051)	0.898* (.104)

A. Values in the Table are mean ± (standard deviation), and * indicates statistically different from Day 1 group ANOVA $p<0.05$

The information shown in Table 3 illustrates a dose dependent decrease in calcium incorporation into the shell. This effect could be due to several factors: (1) a direct effect on the shell gland and mantle, (2) the enzymes involved in shell deposition (3) an alteration in calcium adsorption through the digestive tract due to lesions (see Table 4) or some other unknown mechanism.

TABLE3. 2,3,7,8-TCDD effect on Calcium-45 deposition into the shell matrix of *Mya arenaria*[A]

	Treatment Group (nominal concentration in ug/L)[B]					
	Control	Nonane	2.5	25	250	2,500
Calcium-45 Levels DPM	1398 ± 423	1140 ± 263	1020 ± 217	949 ± 256	970 ± 220	876 ± 260

A. The animals were sacrificed 21 days post 2,3,7,8-TCDD treatment and seven days post calcium-45.
B. Significantly different from no treatment control ANOVA $P<0.05$.

Mya arenaria did demonstrate a number of histological effects in various tissues following exposure to 2,3,7,8-TCDD dissolved in nonane. The appearance of the lesions were both time and dose dependent. The lesions were observed in epithelial tissues. The lesions observed in these tissues included: necrosis, severe vacuolization, hyperplasia and metaplastic changes. No lesions were observed in the gonadal follicles even though they were a major site for 2,3,7,8-TCDD accumulation. This may have been due to the fact that the gonads were fully developed and were not undergoing differentiation. Effects were observed at the lowest dose tested 2-2.5 ng/L for 24 h, which would result in a tissue dose of approximately 5-10 pg/g. Similar lesions have been observed in Newark Bay clams with similar tissue doses.[5] Elimination of 2,3,7,8-TCDD does occur when the clams are transplanted to non contaminated areas.[6]

TABLE 4. Moderate to severe lesions in *Mya arenaria* following water-borne exposure (24 h) to 2,3,7,8-TCDD dissolved in nonane^

TISSUE	EXPOSURE CONCENTRATION ng/L (24 H) NONANE	2-2.5	20-25	200-250	2500
KIDNEY	---	---	+++	+++	
GILL	---	---	+++	+++	
MANTLE	---	---	---	+++	+++
INTESTINE	---	---	---	+++	+++
DIGESTIVE GLAND	---	+++	+++	+++	
FOOT	---	+++	+++	+++	

A. (---) indicates no significant difference, (+++) indicates a significant increase in lesions and ( ) indicates not tested.

The results and effects observed in *Mya arenaria* are summarized in Figure 1. The 2,3,7,8-TCDD is filtered through the incurrent siphon (1) and is dissolved in the mucus layer of the gill (2). The mucus and compound are then carried into the digestive tract (3) where the compound crosses the epithelial cells and is distributed to the gonads (4) and to a lesser extent to the mantle gill and kidney. The mantle, kidney and gills are also the major route for elimination of these compounds. Based on the lesions, and tissue distribution studies the effects on shell synthesis and weight loss could be due to decreased nutrient assimilation, feeding efficiency and effects on the mantle's ability to secret shell.

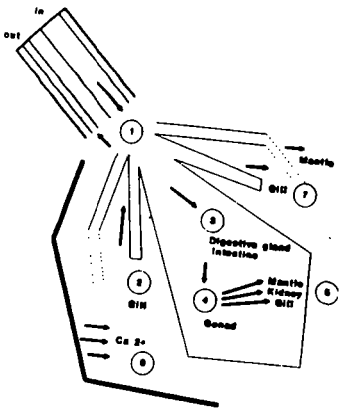

Figure 1. TCDD toxicity and distribution in the Soft-shell clam

4. Conclusions

Invertebrate species are effected by exposure to PCDD/PCDF compounds and are similar to higher vertebrates in that, the effects appear to involve epithelial tissues and tissues which are metabolically active or hormonally regulated. Histological effects are observed at tissue dosages in the range of 5-10 pg/g wet weight of 2,3,7,8-TCDD. This is in agreement with lesions observed in field population in Newark Bay where 2,3,7,8-TCDD levels were observed between 5-20 pg/g wet weight and similar lesions to those produce in the laboratory were observed.[2] There was no evidence of vascular effects and this is probably due to the open circulatory system in most invertebrates. Further studies need to be carried out to better characterize these effects with specific target tissue doses and the biochemical effects on hormonal related changes.

5. References

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