Executive Summary For Studies Carried Out On The Distribution Of Dioxins and Furans Within the Biota and Dose Related Effects

The studies described below were carried out by a number of researchers, and students and they are listed below. These studies were a collaborative effort and the funding was through grants from the NJDEP Division of Science and Research C29433 and P30992.

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The following Specific Aims were addressed in this research. All of the specific aims were met or exceeded as outlined in the grant.

1) Studies were carried out that determined the extent of isomer specific dioxins and furans present in various trophic levels in Newark Bay and Tuckerton, N.J.

2 & 3) Transplant studies (clean to dirty, dirty to clean) were carried out that characterized the histological effects and the toxicokinetics of uptake and elimination from Mya arenaria.

4) Laboratory studies were carried out examining the toxic effects and distribution of TCDD in both Mya arenaria and Callinectes sapidus.

5) Studies were carried out examining a number of isomers for their effects on developing fish embryos and establishing the structure activity relationship.

6) Studies were begun to examine the effects of dioxins on the neuroendocrine and reproductive physiology of the blue crab, Callinectes sapidus.

Studies have been carried out to examine the levels of dioxins and furans in aquatic animals inhabiting the Passaic River, Newark Bay and Raritan Bay. The studies involved both field and laboratory experiments designed to determine the levels of dioxins in various trophic levels and whether these levels posed any adverse effects to the organisms. The results from these studies have shown that this estuary is heavily impacted by this class of compounds. The wide distribution of a number of the isomers would indicate a number of potential sources for these compounds. This is based on principle component analysis and industrial inventories carried out by the NJDEPE. The principle component analysis failed however to correlate concentration into the second phase of the evaluation. The principle component only evaluated the presence or absence of any isomer and did not weight the concentration. If the concentration component was factored into the study the primary source would be off 80 Lister Avenue. Based on sediment cores and levels of the 2,3,7,8-TCDD found in animals near the 80 Lister Avenue site there is no question that the major contributor of this isomer is from this single point source. This specific isomer is the most toxic and will bioaccumulate into higher trophic levels. The sediment contamination from this location has resulted in the contamination of sediments throughout the Newark Bay and into Raritan Bay. The migratory patterns of estuarine species both commercial and forage species has resulted in widespread contamination of the biota. Based on laboratory studies using pure TCDD and natural food (Mya arenaria) the levels which are currently present in these species pose a serious adverse effect to animals living in these waters. This class of compounds effects the developing organism and at the reported levels would reduce larval and embryo survival. The concentrations that would have been present based on historical core samples would have resulted in dramatic decreases in fecundity and larval survival. Studies are desperately needed to follow the concentrations in these animals and their ability to successfully reproduce. In addition, there is a need to examine human populations who use this estuarine system for a source of food and the potential effects on their offspring. These compounds accumulate in human fat and are mobilized to breast milk where the developing child is exposed. Correct studies have demonstrated that these compounds do effect biochemical and morphological parameters in mammals. It must be realized that these compounds probably only represent the tip of the iceberg for lipophilic compounds that are present in human fat and breast milk. The studies which were carried out are summarized below and the references for the published information is also supplied.

The trophic level studies demonstrated that in both plants and invertebrate species that both the 2,3,7,8- and non 2,3,7,8-substituted isomers are present in the Arthur Kill. The plants and algae have the lowest levels followed by the annelid worms, shrimp and bivalve molluscs. The forage fish have both the 2,3,7,8-dioxins and the 2,3,7,8-furans but the furans are not accumulated to the same level as the TCDD. In the game fish that were examined the most toxic isomers were the isomers present in the tissues and the fillets. The fillet is generally at about 1/10 that of the liver or the adipose tissue. The gonads were also a tissue that had high concentrations of the 2,3,7,8-TCDD. This again raises the question on the effect of the developing egg and larvae and the effect of recruitment. Based on the laboratory studies discussed below the levels in the gonads are at high enough levels to elicit toxic responses in the offspring. Again future studies should examine the effects on steroid metabolism in the adult and early developmental stages.

The major finding from these studies was that the chemically impacted sub population of Fundulus heteroclitus (Newark Bay) as compared to a non-impacted sub-population (Tuckerton) were resistant to effects of 2,3,7,8-TCDD . The resistance was observed in two lifestages (embryonic and adult) and on a biochemical level (P450 1A activity as measured by EROD). The significance of this is that these fish have a very small home range and the fish in Newark have been exposed for many generations to TCDD. Because of this stressor we believe they have been genetically selected to be resistant to TCDD. In insect and other fish species it has been shown that exposure to a stressor for 8 to 10 generations will result in a genetically resistant group of animals. It is our hypothesis that this is a similar situation for the F. heteroclitus which live in Newark Bay. This resistance would not be observed in migratory fish species or species who migrate through the area because they are not continually exposed. Fish as with other organisms will eliminate dioxins and furans with t 1/2 of elimination for .5 to 6 months with larger fattier fish eliminating at a slower rate.

The Newark fish did not respond to TCDD treatment in any of the following categories: death, liver to body weight ratio, and lesion occurrence. The Newark fish did exhibit a greater parasite infestation of the liver, hepatic neoplasms and other histological lesions when compared to Tuckerton fish.

The Tuckerton fish exhibited TCDD-related lesions, and significant increases in liver to body weight ratios when exposed to TCDD. Compared to other fish species F. heteroclitus is one of the most resistant to toxicity. The LD50s for a number of fish species is in the range of 3 to 16 ng/g TCDD, while for the Tuckerton fish it was between 30 to 60 ng/g. In the Newark fish there was no effect observed even at the highest dosages examined.

There was no difference between the Newark and Tuckerton fish for uptake, metabolism or elimination. Because there were no differences for these parameters it is hypothesized that the differences are due to an alteration in the receptor or DNA responsive elements. The lack of inducibility in the Newark fish and the ability to induce in the Tuckerton fish would support the alteration in the Ah-receptor or the related induction cascade.

Prince, R., 1993. Comparison Of The Effects of 2,3,7,8-Tetrachlorodibenzo-p-dioxin on Chemically Impacted and Nonimpacted Subpopulations of Fundulus heteroclitus. Dissertation, Rutgers University, p. 231.

Using the Japanese medaka (Oryzias latipes) as a surrogate species for extrapolation to other embryonic effects in fish a number of important observations have been made concerning the effects of TCDD on developing fish embryos. The Japanese medaka is a good surrogate because of it's clear chorion and ability to be collected throughout the year. This enables testing all year long as opposed to testing with Fundulus heteroclitus, which is limited to 3 months. The Japanese medaka is in the same Family (toothcarp) as Fundulus.

Based on levels of radioactivity recovered from dechorionated embryos the ED50 for lesions is 0.240 pg of TCDD equivalents/mg of embryo weight. The toxic equivalent factors (TEFs) that have been developed for mammalian systems are different from those developed for fish. In the fish embryos the toxicity of 2,3,7,8-TCDD is similar to 2,3,7,8-TCDF and 1,2,3,7,8-PCDD, while in mammals both of these isomers are 1/10 to 1/100 as potent as TCDD. The 1,2,3,4,7,8-HCDD, 1,2,7,8-TCDD and 2,3,7,-TriCDD were only slightly toxic and only at very high concentrations. The concentrations were so high that they were not environmentally relevant.

TCDD was shown to be stage specific in it's toxicity, which would suggest that there is a critical protein or other component that is being produced at this time. In these fish the stage where lesions begin to appear is during liver development. It was also shown that P450 could be detected in the developing embryo and this corresponded with liver formation. The induction and the toxicity were shown not to be directly related but are endpoints that indicate TCDD exposure.

The data presented in these studies on the progression of both gross lesions and lesions observed at the light microscopy level suggest that the primary target is the cardiovascular system. This includes the vasculature itself, the heart and the developing blood cells. TCDD is known to alter the normal differentiation of tissues and this alteration in the developing embryo is probably the cause of the lesions. One of the most striking findings was that the RBCs were not differentiating properly and were in some cases two times larger than normal RBCs. There were effects on the endothelial cells lining the vasculature, which could explain the edema that is observed in these embryos. This effect on differentiating tissue may explain in part why invertebrates are less sensitive to TCDD, because of their more open circulatory system and less dependence on closed vasculature system.

Wisk, J., 1990. The Mechanism of Stage Specific toxicity of 2,3,7,8-Tetrachlorodibenzo-p-dioxin In The Embryo of The Japanese medaka (Oryzias latipes). Dissertation, Rutgers University. p. 177.

The research on the natural populations of soft-shell clam from Newark/Raritan Bay estuary have shown that clam populations closest to the point source of TCDD contamination in Newark Bay have the highest concentration (11-20 ppt wet weight) of TCDD. Animals collected from the Arthur Kill ranged from 6.3-10 ppt, while animals collected from the farthest point from the source had levels between 0.5-1.1 ppt.

The soft-shell clam is a good animal to monitor in order to determine the pattern in the suspended sediment. Since the pattern of PCDD and PCDF isomers observed in the Arthur Kill clams is similar to that found in the sediments and suspended sediments, the soft-shell clam can be utilized to identify sources of contamination.

The levels of TCDD in clams from the Arthur Kill did not decrease from 1986 to 1991. The configuration of the rivers and bays may result in trapping of sediments and contribute to the apparent consistent levels of TCDD in clams. The levels of TCDD in the suspended sediments have remained constant over the study period of about 100 pg/g. This would suggest that the Bioacummulation Factor from Sediment (BAFS) is on the order of 0.1. This value is consistent with values being reported from the Great Lakes where the BAFS indicate that steady state levels are below 1.0 when compared to sediments. What this indicates is that the soil or sediment matrix plays an important role in the amount of compound that is able to be absorbed by any organism.

The levels of TCDD and TCDF found in the populations of clams obtained from Tuckerton, NJ and the Chesapeake Bay were much lower than those in the Newark Bay and Arthur kill area populations. TCDD levels were below 1 ppt in all samples from Tuckerton and the Chesapeake Bay, while the TCDF levels exceeded 1 ppt on only one occasion at Tuckerton. The fact that detectable levels of TCDD and TCDF were present is consistent with the world wide occurrence of these contaminants. Because of the low background levels these sites were considered the control sites.

In two separate transplant studies it was demonstrated that control clams transplanted into the Arthur Kill quickly bioaccumulated dioxins and furans within the first month after transplant. By three months post transplant the clams had reached levels of TCDD and TCDf which were comparable to those in the natural Arthur Kill population. Collection of clams throughout the year did show that there were fluctuations in the levels, but the transplanted and natural clam populations were similar. The asiatic clam proved to be useful for transplanting into freshwater while the soft-shell clam worked well for the estuarine environments.

When clams were transplanted from the Arthur Kill to a clean environment in Tuckerton, NJ the clams eliminated more than 69% in the first month. By four months post-transplant the clams were well within the range of TCDD and TCDF levels which were found in the natural Tuckerton population. At the 10 month time point 99%

of the dioxins and 90% of the furans had been eliminated. The lower percentage elimination for the TCDF may reflect atmospheric input into the estuarine system.

These studies demonstrated three major points 1) that TCDD and TCDF are bioavailable in the Arthur kill, 2) the soft-shell clam will accumulate both 2,3,7,8-isomers as well as non-2,3,7,8-substituted isomers. and that soft-shell clams are able to eliminate TCDD and TCDF and other congeners after transplant to a non-impacted environment with an estimated half-life for TCDD of 35 days and for TCDF of 30 days.

A physiologically based pharmacokinetic model (PBPK) for TCDD was developed for the bivalve mollusk _Mya arenaria_. This task was carried out to develop a PBPK model which could be used as a predictor of bioconcentration of these lipophilic compounds. _Mya arenaria_ was selected because it is an important food base in the Newark/Raritan estuary system. In addition, bivalve mollusks accumulate the same pattern of isomers that are present in the sediments in which they are exposed. The conclusions that were drawn from these studies are listed below.

1. The PBPK model for _Mya arenaria_ predicts the absorption, distribution and excretion of TCDD as a function of lipophilicity and the relative lipid content of the model compartments.

2. The model was validated with laboratory and field studies which gave very good correlation between predicted and observed values.

3. The initial distribution of dioxin in the compartments reflected the differential perfusion rates and lipid content. Redistribution to the gonads at steady state results in the highest TCDD dose in the gonadal regions.

4. Steady state is predicted to be 20 days and the t 1/2 for elimination is 3 days under continuous filtration conditions. These values are shorter than those observed in the transplant studies (see above), but when more conservative filtration times and rates are used there is good agreement.

The PBPK model for _Mya arenaria_ can be applied to investigate and predict the concentration of dioxins and other persistent lipophilic compounds in bivalve mollusks under different environmental conditions. It can be used in risk assessment, as well as in studies involving bioconcentration and bioaccumulation of xenobiotics in natural environments. Another application of this model is to be coupled to other environmental transport and fate models, and other PBPK models for other organisms. This would enable an estimation of the movement of a compound through an ecosystem.

Moreno, M., 1991. A Physiological Based Pharmacokinetic Model For 2,3,7,8-Tetrachlorodibenzo-p-dioxin In Bivalve Mollusks. Masters Dissertation, Rutgers University, p. 83.

Clams collected from the Newark Bay and the Arthur Kill estuary had higher incidence of lesions in all organs examined except the gill and heart when compared to the Tuckerton and Chesapeake Bay populations. The lesions from the naturally occurring clams could not be specifically determined as to the etiologic agent because of the complexity of chemicals present in this water system. The clams from the Arthur Kill had distinct and unique lesions which were not observed in Tuckerton or Chesapeake clams, had lesions which were consistent with chemical insult rather than a developmental or parasitic effect and lesions that could impair shell deposition. Clams when transplanted from the Arthur Kill to a cleaner site there was a promotional effect of the lesions, which resulted in initially more severe lesions than animals maintained at the Arthur Kill site. Tuckerton clams transplanted into the Arthur Kill resulted in similar lesion being observed at the end of 1 month and later. In a series of laboratory studies in which the clams were exposed to pure TCDD similar lesions observed in the field clams were reproduced in the laboratory. The shell thinning and lesions of the digestive tract were very similar to those observed in the field. Based on these findings it is likely that a number of the lesions observed in the naturally occurring clams are due to TCDD. A study carried out using Ca45 revealed that TCDD could dramatically alter the depositional rate both at the edge of the shell along the inside. There need to be further studies into the interaction of these other pollutants and the dose relationship between lesions. The distribution studies carried out in the laboratory showed that the gonads were an organ which sequestered the dioxin and this could result in the eggs being released being exposed to high concentrations of TCDD. Since the developing organism is the most sensitive this could result in effects on the developing larvae. This is another area that needs further research.

Brown, R., 1991. The Toxicokinetics and Histological Effects Of 2,3,7,8-Tetrachlorodibenzo-p-dioxin on the soft-shell clam, *Mya arenaria*. Dissertation, Rutgers University. p. 222.

Studies were carried out examining the distribution of TCDD in blue crabs fed clam-jello and the accumulation of TCDD into various tissues. In a similar fashion as seen in the field the TCDD accumulated in the hepatopancreas (HP) to the greatest extent and to about 1/10 of the HP level in the muscle. The levels of TCDD could be monitored by taking blood samples from the leg joint. This technique was a very efficient way to dose the blue crabs and obtain tissue doses similar to field levels. In another set of studies the crabs were fed clams from Newark Bay and a delay in molting was observed as were fewer molts and similar results were obtained using TCDD spiked food. Based on these preliminary studies TCDD does effect the molting cycle and the growth of rapidly molting blue crabs. Future studies should focus on understanding the mechanism by which these effects are caused and on the crab larvae from dioxin contaminated animals. These studies in collaboration with Dr. Rao could indicate a basic mechanism of TCDDs effect on this important commercial species. It must be emphasized that the adult crab is not stage of most concern, but it is the larvae and rapidly developing organism.

Dr. Rao developed a enzyme-linked immunosorbant assay (ELISA) for ecdysteroid in the hemolymph of blue crabs. This assay was very sensitive and was able to demonstrate that as the crabs went through various molt stages that the B-ecdysone levels varied. The ED50s were about 3 fmol/microwell with a useful range of 0.5 to 20 fmol/microwell. The ecdysteroid values for crabs ranged from 0.4 to 43 ng/ml, which is in good agreement with published data. The amount of hemolymph needed for this assay is less than 500 ul, which can be obtained from small developing crabs. Dr. Rao was able to localize B-PDH in the blue crab central nervous system using immunocytochemistry. In addition, the primary structure of PDH from the sinus gland was carried out and was identical to the sequence in other brachyurans: NSELINSILGLPKVMNDA. Dr. Rao's group also purified vitellin from the blue crab ovaries so as to prepare antiserum for ELISA and immunochemistry. These techniques would allow for the determination of the effects of dioxins on the endocrine system and vitellogenesis.