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REVIEW ARTICLE

Assessing the Chemical Sensitivity of Freshwater Fish Commonly Used in Toxicological Studies

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Using information from published studies, the relative sensitivity of various freshwater fish to a range of chemicals was examined. Specifically, the objectives were to: (1) determine which species are used most often in toxicity tests, (2) assess the relative sensitivity of these species to various chemicals, and (3) determine whether the two most commonly tested species exhibit differences in their relative sensitivity to different classes of chemicals. Fathead minnows, rainbow trout and bluegill sunfish were the three most commonly used species in 96-h LC50 tests. Of the nine species examined, coho salmon and rainbow trout were the most sensitive species to 190 chemicals, while goldfish and carp were the least sensitive. Fathead minnows and rainbow trout were not equally sensitive to 13 different classes of chemicals; for example, while trout were significantly more sensitive to metals, fathead minnows were more sensitive to hydrocarbons. Such comparisons are expected to be useful for predicting the relative responses of different species to previously untested chemicals in such groups, and in gaining insight into physiological modes of action.

Key words: **chemical sensitivity, toxicity tests, rainbow trout, fathead minnow**

Introduction

Model species are organisms used by biologists to provide general information about living systems and processes. These species typically exhibit a body plan and physiology that is reasonably nonspecialized so that information obtained from their examination can be used to generalize about other groups. A variety of such organisms has been used to investigate the toxicity of chemicals in an effort to understand their potential effects on both human and environmental health. As many of these substances, or their breakdown products, end up in rivers, lakes and oceans, it is important to study model species that suitably represent the fauna of these habitats.

A variety of fish species is currently used to provide information about the toxicity of chemicals, including rainbow trout (*Oncorhynchus mykiss*), fathead minnows (*Pimephales promelas*) and bluegill sunfish (*Lepomis macrochirus*). However, the degree to which these species differ in sensitivity to various substances is not well established. Species-specific sensitivity can occur for a number of reasons, including differences in the amount of chemical that enters the organism, how it moves around the body, and how it is metabolized and excreted (Grue et al. 2001). Information concerning species sensitivity is important in toxicological studies, particularly when trying to form generalizations and guidelines concerning the use of specific chemicals based on studies of one or a few species. Species-specific responses to certain chemicals may also have important ecological consequences, particularly if they result in altered community structure. Hansen et al. (1999), for example, suggested that differential sensitivity to metal contamination was responsible for a change in the relative proportions of rainbow trout and brown trout (*Salmo trutta*) in different sections of a Montana river.

Constraints imposed largely by time and funding have resulted in the vast majority of studies being carried out using single species as a model organism. In a limited number of investigations, species representing a range of taxonomic groups have been used to assess the toxicity of a certain substance. For example, Nebeker et al. (1989) examined the sensitivity of a cladoceran, two amphipods, an annelid worm and the fathead minnow to hexachlorobenzene. Multi-species investigations examining the chemical sensitivity of fish have typically compared two (Routledge et al. 1998), three (Bengtsson et al. 1988) or more (Elonen et al. 1998) species to a single chemical or chemical mixture. It remains unclear whether such sensitivity differences are consistent across a broad array of chemicals.

The first objective of this study is to determine which freshwater fish species are most commonly used to assess the toxicity of chemicals. The second objective is to examine the relative sensitivity of these species to a

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number of different chemicals. The final objective is to test whether relative sensitivity is affected by the category of chemical being examined using two of the most commonly tested species.

Materials and Methods

Data Set

All data for this project were obtained from the ECOTOXicology Database System (www.epa.gov/ecotox/) provided by the United States Environmental Protection Agency as a public service. This database provides information about the effects of individual chemicals on aquatic and terrestrial life, compiled primarily from peer-reviewed literature. Due to the large number of studies from which the data were taken, references to individual sources have not been included in this review. Information in the database includes test results (endpoints, effects and measurements) and test conditions (location, media, exposure type) for various aquatic and terrestrial species exposed to a wide range of chemicals. Some limitations of the ECOTOX data set and search engine are discussed on the website; other limitations that pertain to our analysis are the absence of age-specific or size-specific information for each fish species. Within a fish species, the grouping of various fish age groups by the database does not allow the variables of age or size of the fish to be examined.

Species Selection

Our first objective was to determine the fish species most commonly used in toxicity testing. To narrow our search parameters, it was decided to examine only those studies that presented LC50 (96 h) values for the species and chemical being examined. In addition, our search was limited to studies published since 1980. Note that a few of these studies (Johnson and Finley 1980; Office of Pesticide Programs 2000) are reviews of other toxicity investigations and thus some information may predate 1980. This search provided a total of 3119 LC50 values for 12 different species exposed to various chemicals. The three most commonly tested species were fathead minnows (930), rainbow trout (827) and bluegill sunfish (743). These were followed by guppies (*Poecilia reticulata*; 150), carp (*Cyprinus carpio*; 114), goldfish (*Carassius auratus*; 96), coho salmon (*Oncorhynchus kisutch*; 80), largemouth bass (*Micropterus salmoides*; 59), mosquitofish (*Gambusia affinis*; 58), mummichogs (*Fundulus heteroclitus*; 22), Japanese medaka (*Oryzias latipes*; 21) and three-spine sticklebacks (*Gasterostreus aculeatus*; 19).

Species Comparisons

From these data, a subset of chemicals was selected to be used for species comparisons. This included any chemical for which LC50 information was available for both fathead minnows and rainbow trout, the two most commonly tested species. It was assumed that this list would contain most of the chemicals that were likely to be tested using one or more of the other species as well. The total number of chemicals that fit this criterion was 296.

Lastly, any studies for which direct comparisons between species were not legitimate were eliminated. This typically meant ensuring that the experimental setup and purity of the chemical were consistent for the species being compared. For example, the few studies that were conducted in saltwater were eliminated. In addition, only studies in which both used either a flow-through or static exposure system were compared. As chemicals tested varied substantially in terms of purity, pairs of species might have more than one comparison for any one chemical. For example, rainbow trout and fathead minnows were both exposed to pentachlorophenol at purities of 88, 95 and 99%, providing three comparisons for this chemical. Any chemical for which no information was provided on the grade or purity was discarded unless the species were being compared in the same study; in these cases, it was assumed that the same chemical purity was used for each.

Chemical Classification

There were 190 chemical tests that provided comparable LC50 data for both fathead minnows and rainbow trout. Many chemical tests provided LC50 data for some other species as well. Of these 190 chemicals, it was possible to classify 146 chemicals into 13 chemical classes based on chemical structure (Table 1). Some of the 146 classified chemicals had more than one rainbow trout to fathead minnow LC50 ratio, and some chemicals had several LC50s as they were tested several times, either in separate studies or with different chemical purities. In these cases, the arithmetic mean of the trout to fathead LC50 ratio was used. The 44 chemicals that were unclassified by our system were occasionally included with the data from the classified chemicals and used in comparisons of "all chemicals."

Statistical Analyses

To determine if species exhibited differences in sensitivity from rainbow trout or fathead minnows (see Results), t-tests were used with a Bonferroni adjustment to correct for multiple comparisons. All statistics were performed using Systat (Evanston, Ill.). A similar procedure was used when examining differences in the sensitivity of trout and fatheads to different groups of chemicals. The number of comparisons made in each assessment is shown in the data (the numbers below the bars in Fig. 2 and 4, and the numbers above the bars in Fig. 3), with asterisks indicating differences that were statistically significant ($P < 0.05$).

TABLE 1. Chemical classification for data used to compare LC50s for fathead minnows and rainbow trout

Chemical class	N	*General description of chemicals included in class*
Metals	7	Metal salts of Ag, As, Cd, Cu, Hg
Hydrocarbons	21	Hydrocarbons with Cl, N, O, OH or S groups
CH-chains	5	Chain hydrocarbons, some with OH groups
Benzenes	23	Benzene rings with Cl, F, N, OH (some biphenyls and anilines)
(Poly)aromatic hydrocarbons	9	1–4 ring (P)AH, some with CH groups
Phenols	6	Phenols, diols, some with CH groups
Triazines	6	Triazine herbicides
Pyradines	6	Compounds based on picloram
Organochlorines	15	Compounds such as DDT, lindane, dieldrin, methoxyclor, toxaphene, chlordane
Organophosphates	23	Compounds such as malathion, parathion, clorpyrifos, phosmet
Carbamates	9	Compounds such as carbofuran, aldicarb, carbarly, methomyl
Phthalates	12	Diethyl-, dibutyl- and benzyl-phthalates
Pyrethroids	4	Compounds such as allethrin, fenvalerate

Results

Species Comparisons

To provide relative comparisons, information obtained from exposures involving rainbow trout for baseline data was used. For example, there were 259 comparisons from 190 chemicals meeting the criteria described in the Methods section between rainbow trout and fathead minnows. The relative sensitivity of the species was compared by plotting the log(trout LC50)/fathead LC50 (Fig. 1). Values of this ratio falling below 0 indicate that rainbow trout are more sensitive for that chemical while those above 0 are indicative of greater fathead sensitivity. Because different concentrations or purities of the same chemical cannot be considered independent, the mean of all such values was used, resulting in a total number of comparisons of 190 (equal to the total number of chemicals).

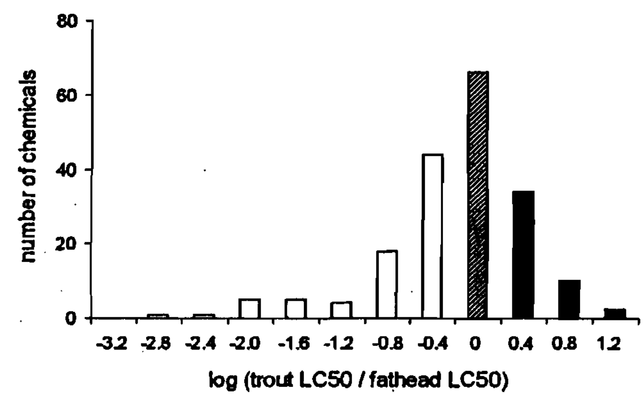

Fig. 1. Frequency distribution of the log of the ratios of rainbow trout LC50s to fathead minnow LC50s for 190 chemicals. Values above zero (black bars) indicate the number of chemicals to which fatheads were more sensitive while those below zero (white bars) indicate the number to which trout were more sensitive. Hatched bars (from -0.2 to +0.2) refer to chemicals to which both species exhibit relatively similar sensitivity.

Rainbow trout exhibited greater sensitivity to 138 of the 190 chemicals while the two species were equally sensitive to six. It should be emphasized that most values fell between -1 and +1, indicating sensitivity differences between the two species were usually small (a value of 1 represents a 10-fold difference in sensitivity). Overall, this analysis suggests that fathead minnows are about 42% as sensitive as rainbow trout to this group of 190 chemicals.

Similar analyses were done for each of the other species (Fig. 2). Japanese medaka, mummichogs and sticklebacks are omitted from further examination as

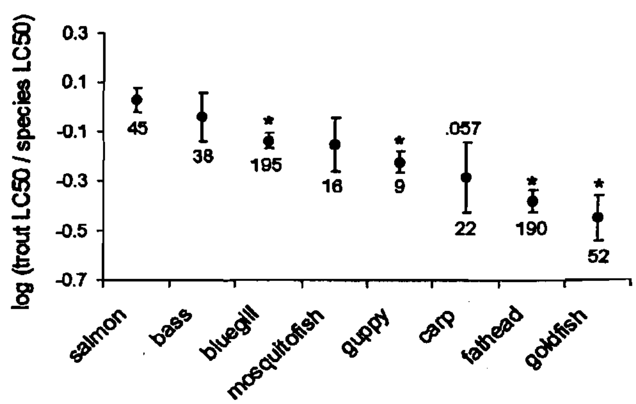

Fig. 2. Log of the ratio of 96-h LC50s for various chemicals in rainbow trout to the 96-h LC50s in eight different species, to allow for sensitivity comparison among the species most commonly used in LC50 tests. To assess relative sensitivities, rainbow trout were used as the "baseline" species, and all other fish were compared to them. Thus, a value of zero indicates the same overall degree of sensitivity to the chemicals as was found with trout. Shown are means ± standard errors. Numbers below each mean are the number of comparisons between that species and rainbow trout. Asterisks indicate significant differences from trout ($P < 0.05$). For the comparison of carp and trout, the P value of 0.057 is shown above the bar for carp, as it was marginally significant.

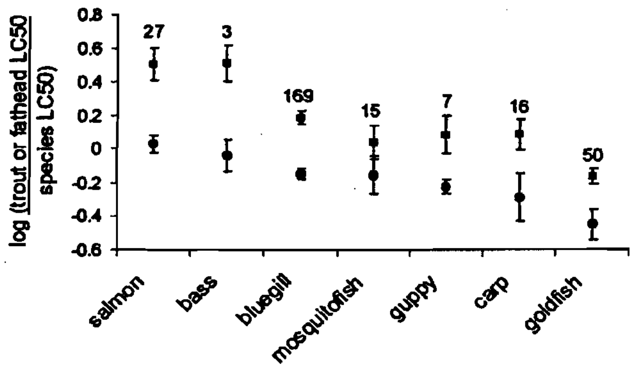

Fig. 3. Log of the ratio of 96-h LC50s for various chemicals in fathead minnows (squares) to the 96-h LC50s in seven different species, to allow for comparison in sensitivity among species most commonly used in LC50 tests. The relative sensitivity of each species is similar to those found when species were compared with rainbow trout (circles; same data shown in Fig. 2). Numbers above points indicate the number of comparisons between that species and fathead minnows. Sample sizes for rainbow trout are provided in Fig. 2.

they contained too few legitimate comparisons with rainbow trout. In addition to fathead minnows, bluegill sunfish, guppies and goldfish were all significantly less sensitive to the acute effects of these chemicals than were rainbow trout. The values for mosquitofish and carp fell within the ranges of these species but did not differ significantly from trout because of a smaller sample size and/or higher degree of variation. The least sensitive species was goldfish, having a sensitivity of about 36% that of rainbow trout. The most sensitive species, coho salmon, did not differ significantly from rainbow trout.

To determine if the ranking of species sensitivity for this group of chemicals was consistent, data were also compared with fathead minnows (Fig. 3). The relationship between species was the same with the exception of mosquitofish and guppies which switched rankings. However, values for these two species did not differ from one another in comparisons with either trout or fatheads (t-tests, $P > 0.05$). Thus, it would appear that these rankings are relatively robust.

Sensitivity to Different Chemical Compounds

The objective of our final analysis was to determine if the relative sensitivity of rainbow trout and fathead minnows (two of the most commonly used species in toxicological studies) exhibited variable sensitivity to different classes of chemicals (phenols, benzenes, pyrethroids, etc.). Data from the 146 chemicals used for this analysis suggest that the two species do indeed exhibit different sensitivity to the 13 classes (Fig. 4). For example, while trout were almost 10 times as sensitive to metals, the two species exhibited equal sensitivity to CH-chains and phthalates, and fathead minnows were significantly more sensitive to hydrocarbons.

Discussion

Our objectives in this study were to determine the freshwater fish species most commonly used in acute toxicity testing, to determine if these species differed in sensitivity to a range of chemicals, and to ascertain whether sensitivity differences were consistent across a range of compounds. The wide variety of endpoints used in toxicology studies made it difficult to determine the most commonly used species. To simplify our search, only those species which have been used most often to provide LC50 values over 96 h since 1980 were selected. Using this criterion, fathead minnows, rainbow trout and bluegill sunfish emerged as the most commonly tested fish. Clearly, however, certain species are used more commonly in other types of studies. Japanese medaka, for example, have been used extensively in testing the teratogenic properties of certain substances (Yamamoto 1975 cited in Loosli et al. 2000; Hawkins et al. 1995, 1998; Ishikawa 2000) and LC50 tests are not typically used in such investigations. Recognizing these limitations, it was expected that our list of species contained most of the commonly tested freshwater fish and that minor discrepancies would not affect the more important second and third objectives.

The results of this study suggest that there are statistically significant differences in chemical sensitivity among freshwater fish commonly used in toxicology studies. There was a threefold difference in sensitivity between the most and least sensitive species, based on the

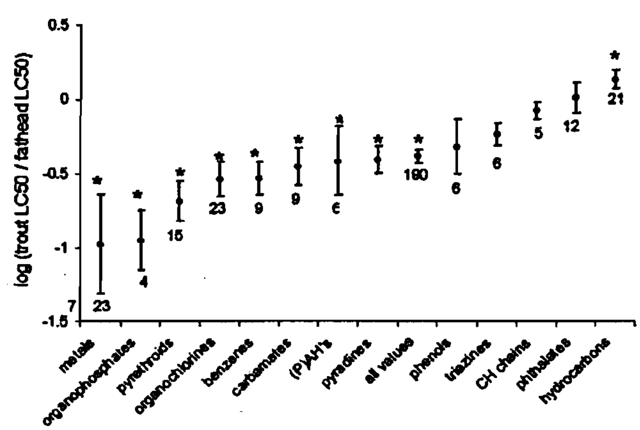

Fig. 4. Log of the ratio of 96-h LC50 in rainbow trout to the 96-h LC50 in fathead minnows, for chemicals grouped into thirteen chemical classes, based on structural formula. A value of zero indicates that the two species were equally sensitive to that group. Numbers below points are the total number of comparisons between the two species for each chemical group. Asterisks indicate significant differences between the two species ($P < 0.05$).

subset of 190 chemicals used in our analysis. The two most commonly tested salmonids, coho salmon and rainbow trout, were the most sensitive while the two cyprinids, carp and goldfish, along with fathead minnows, were the least sensitive. Whether these differences are biologically meaningful depends largely on the question being addressed. Typically, multiple species comparisons differ by 2 to 3 orders of magnitude although this may be as great as 4 to 5 orders of magnitude (Schäfer 1994; Eisler 2000). In assessing the lethal potency of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), Poland and Knudsen (1982) noted a 3000-fold difference in sensitivity among laboratory mammals. The seven species of fish exposed to the same substance exhibited substantially less variability in response, although the 5-fold difference observed was still greater than that found in our analysis.

Few attempts have been made at broad generalizations concerning interspecific differences in relative sensitivity. Vaal et al. (1997) found that the largest interspecies variation in sensitivity was for compounds with the highest overall toxicity in a comparison of the acute toxicity of 21 compounds in 26 aquatic invertebrate, fish and amphibian species. In addition, saltwater fish species were more sensitive (had lower EC50s) than freshwater fish species for 11 of 22 chemicals (Hutchinson et al. 1998). Saltwater and freshwater species were of equal sensitivity for 6 of 22 chemicals, and freshwater species were more sensitive than saltwater species for 5 of 22 chemicals (Hutchinson et al. 1998).

Within species variability in response to toxic substances is also well documented. Often such variability reflects differences in sensitivity of different life stages. Early life stages (eggs and fry) are typically found to be more sensitive to chemicals than later stages (McKim 1977, 1985; Birge et al. 1985). For example, larvae of three endangered fish species (Colorado squawfish [*Ptychocheilus lucius*], bonytail [*Gila elegans*] and razorback sucker [*Xyrauchen texanus*]) are more sensitive than juvenile stages to cadmium, hexavalent chromium and mercury (Buhl 1997). Early life stages of fish are among the most sensitive vertebrates to TCDD-induced mortality. The LD50 of TCDD in rainbow trout sac fry (0.4 µg/g egg weight) is about 25 times less than that in juvenile trout (10 µg/g body weight) (Kleeman et al. 1988; Walker and Peterson 1991). However, the relative sensitivity of different species is not necessarily the same for different age groups. For example, the most extensive comparative information for responses of freshwater fish to a single chemical comes from studies on the toxicity of TCDD. Egg exposures of 10 different species, from 5 separate studies (Elonen et al. 1998; Walker and Peterson 1994; Walker et al. 1991, 1992, 1994) suggest that the three salmonids are most sensitive (lake trout [*Oncorhynchus namaycush*], brook trout [*Salvelinus fontinalis*] and rainbow trout). However, when the young of 6 species were exposed to the same chemical, rainbow trout ranked fourth in sensitivity, behind yellow perch (*Perca flavescens*), carp and brown bullhead (*Ictalurus nebulosis*). The principle weakness of the ECOTOX database, and subsequently our results, is the absence of age-specific information. The grouping of various age groups no doubt masked some of the variability in sensitivity that may exist when this variable is controlled.

Due to the substantial inter- and intra-specific variation in response to particular chemicals, Cairns (1986) suggested that the "most sensitive species" concept is a myth. The data here suggest that the benefit of a comparative approach is in the information acquired concerning the relative sensitivity to specific chemical classes. For example, based on the database used, rainbow trout are about 10 times more sensitive to metals than are fathead minnows; however, fathead minnows are significantly more sensitive to hydrocarbons. Both species are equally sensitive to phthalates. If such trends are consistent, this information may be more valuable in linking mechanisms of action with physiological differences between species.

In summary, there is a significant variability between different species of freshwater fish in response to chemical exposures. The predictive power of the relationship observed, however, is expected to be weak given that variability in responses within species is often greater than that observed between species. However, the broad database currently available permits the analysis of more specific trends, such as responses to specific groups of chemicals. Such information will likely be more useful, both in predicting the relative responses of different species to previously untested chemicals in such groups, and in gaining insight into physiological modes of action.

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References

Bengtsson BE, Bengston A, Tjärnlund U. 1988. Effects of pulp mill effluents on vertebrae of fourhorn sculpin, *Myoxocephalus quadricornis*, bleak, *Alburnus alburnus*, and perch, *Perca fluviatilis*. Arch. Environ. Contam. Toxicol. 17:789–797.

Birge JW, Black JA, Westerman AG. 1985. Short-term fish and amphibian embryo-larval tests for determining the effects of toxicant stress on early life stages and estimating chronic values for single compounds and complex effluents. Environ. Toxicol. Chem. 4:807–821.

Buhl KJ. 1997. Relative sensitivity of three endangered fishes, Colorado squawfish, bonytail, and razorback sucker, to selected metal pollutants. Ecotoxicol. Environ. Saf. 37:186–192.

Cairns Jr J. 1986. The myth of the most sensitive species. BioScience 36:670–672.

Eisler R. 2000. Handbook of chemical risk assessment – health hazards to humans, plants and animals. Volumes 1-3, Lewis Publishers, Boca Raton, Florida.

Elonen GE, Spehar RL, Holcombe GW, Johnson RD, Fernandez JD, Erickson RJ, Tietge JE, Cook PM. 1998. Comparative toxicity of 2,3,7,8-tetrachlorodibenzo-p-dioxin to seven freshwater fish species during early life-stage development. Environ. Toxicol. Chem. 17:472–483.

Grue CE, Gardner SC, Gibert PL. 2001. On the significance of pollutant-induced alterations in the behaviour of fish and wildlife, p. 1–90. *In* Dell'Omo G (ed.), Behavioural ecotoxicology. John Wiley and Sons, Ltd., West Sussex, U.K.

Hansen JA, Woodward F, Little EE, DeLonay AJ, Bergman JL. 1999. Behavioral avoidance: possible mechanism for explaining abundance and distribution of trout species in a metal impacted river. Environ. Toxicol. Chem. 18:313–317.

Hawkins WE, Walker WW, James MO, Manning CS, Barnes DH, Heard CS, Overstreet RM. 1998. Carcinogenic effects of 1,2-dibromoethane (ethylene dibromide; EDB) in Japanese medaka (*Oryzias latipes*). Mut. Res. 399:221–232.

Hawkins WE, Walker WW, Overstreet RM. 1995. Carcinogenicity tests using aquarium fish, p. 421–446. *In* Rand GM (ed.), Fundamentals of aquatic toxicology. Taylor and Francis, Philadelphia, Pennsylvania.

Hutchinson TH, Scholz N, Guhl W. 1998. Analysis of the ECETOC aquatic toxicity (EAT) database IV – comparative toxicity of chemical substances to freshwater versus saltwater organisms. Chemosphere 36:143–153.

Ishikawa Y. 2000. Medakafish as a model system for vertebrate developmental genetics. BioEssays 22:487–495

Johnson WW, Finley MT. 1980. Handbook of acute toxicity of chemicals to fish and aquatic invertebrates. Resource Publ. 137. Fish Wild. Serv., USDI, Washington, DC.

Kleeman JM, Olson JR, Peterson RE. 1988. Species differences in 2,3,7,8-tetrachlorodibenzo-p-dioxin toxicity and biotransformation in fish. Fund. Appl. Toxicol. 10:206–213.

Loosli F, Köster R, Carl M, Kühnlein R, Henrich T, Mücke M, Krone A, Wittbrodt J. 2000. A genetic screen for mutations affecting embryonic development in medaka fish (*Oryzias latipes*). Mech. Dev. 97:133–139.

McKim JM. 1977. Evaluation of tests with early life stages of fish for predicting long-term toxicity. J. Fish. Res. Bd. Canada 34:1148–1154.

McKim JM. 1985. Early life stage toxicity tests, p. 48–95. *In* Rand GM, Petrocelli SR (ed.), Fundamental of aquatic toxicology. Hemisphere, New York.

Nebeker AV, Griffis WL, Wise CM, Hopkins E, Barbitta JA. 1989. Survival, reproduction and bioconcentration in invertebrates and fish exposed to hexachlorobenzene. Environ. Toxicol. Chem. 8:601–611.

Office of Pesticide Programs. 2000. Pesticide ecotoxicity database (formerly: environmental effects database [EEDB]). Environmental Fate and Effects Division, U.S. EPA, Washington, DC.

Poland A, Knudsen JC. 1982. 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin and related halogenated aromatic hydrocarbons: examination of mechanisms of toxicity. Ann. Rev. Pharmacol. Toxicol. 22:517–524.

Routledge EJ, Sheahan D, Desbrow C, Brighty GC, Waldock M, Sumpter JP. 1998. Identification of estrogenic chemicals in STW effluent. 2. *In vivo* responses in trout and roach. Environ. Sci. Technol. 32:1559–1565.

Schafer Jr EW. 1994. Comparative avian toxicology: what is its role in predicting and monitoring the effects of agricultural pesticides?, p. 321–337. *In* Kendall RJ, Lacher Jr TE (ed.), Wildlife toxicology and population modeling: integrated studies of agroecosystems. Lewis Publishers, Boca Raton, Florida.

Vaal M, van der Wal JT, Hermens J, Hoekstra J. 1997. Pattern analysis of the variation in the sensitivity of aquatic species to toxicants. Chemosphere 36:1291–1309.

Walker MK, Cook PM, Batterman AR, Butterworth BC, Berini C, Libal JJ, Hufnagle LC, Peterson RE. 1994. Translocation of 2,3,7,8-tetrachlorodibenzo-p-dioxin from adult female lake trout (*Salvelinus namaycush*) to oocytes: effects on early life stage development and sac fry survival. Can. J. Fish. Aquat. Sci. 51:1410–1419.

Walker MK, Hufnagle Jr LC, Clayton MK, Peterson RE. 1992. An egg injection method for assessing early life stage mortality of polychlorinated dibenzo-p-dioxins, dibenzofurans, and biphenyls in rainbow trout (*Oncorhynchus mykiss*). Aquat. Toxicol. 22:15–38.

Walker MK, Peterson RE. 1991. Potencies of polychlorinated dibenzo-*p*-dioxin, dibenzofuran, and biphenyl congeners, relative to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin, for producing early life stage mortality in rainbow trout (*Oncorhynchus mykiss*). Aquat. Toxicol. 21:229–238.

Walker MK, Peterson RE. 1994. Toxicity of 2,3,7,8-tetrachlorodibenzo-p-dioxin to brook trout (*Salvelinus fontinalis*) during early development. Environ. Toxicol. Chem. 13:817–820.

Walker MK, Spitsbergen JM, Olson JR, Peterson RE. 1991. 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) toxicity during early life stage development of lake trout (*Salvelinus namaycush*). Can. J. Fish. Aquat. Sci. 48:875–883.

Yamamoto T. 1975. Medaka (killifish), biology and strains. Keigaku Publishing Company, Tokyo.

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