Int. J. Environ. Res. Public Health **2004**, *1(2)*, 124-131

International Journal of
Environmental Research and Public Health
ISSN 1660-4601
www.ijerph.org

Effects of Long-Term Exposure of the Red Swamp Crawfish *Procambarus clarkii* to a Mixture of Two Herbicides, 2,4-Dichlorophenoxyacetic Acid and Monosodium Methanearsonate, and Associated Human Health Risks

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Received: 28 February 2004 / Accepted: 30 June 2004 / Published: 30 September 2004

Abstract: 2,4-dichlorophenoxyacetic acid and monosodium methanearsonate are often sold in commercial mixtures. Bioconcentration studies have been performed for each of these herbicides individually, but little information exists concerning long-term exposure to a mixture of these herbicides. The following study examined the uptake of arsenic in crawfish after long-term exposure to this mixture, and the health risks associated with consumption of these crawfish. Bioconcentration and depuration experiments using a 50:50 by concentration mixture of the two herbicides, with and without surfactant, were performed to quantify how much arsenic is concentrated in the edible tissue of the crawfish. Of the three tissues (muscle, gill, and hepatopancreas) sampled hepatopancreas bioconcentrated the highest amount of arsenic. Surfactant significantly reduced this uptake but did not affect bioconcentration of arsenic into other tissues. Surfactant had no effect on depuration of arsenic from any of the tissues. Cooking lowered hepatopancreatic arsenic content, possibly as a result of structural changes in the hepatopancreas. Assessment of the human health risk associated with consuming these crawfish showed an exposure dose at the high end of consumption that was approximately twice the reference dose for arsenic. Cancer risks were averaged at approximately 7 extra tumors in a population of 10,000 and 6 extra tumors in a population of 10,000 resulting from a lifetime consumption of crawfish exposed to the herbicide mixture without and with surfactant, respectively.

Keywords: crawfish, 2,4-dichlorophenoxyacetic acid, monosodium methanearsonate, herbicide, mixture bioconcentration, health risk

Introduction

Both 2,4-dichlorophenoxyacetic acid and monosodium methanearsonate are used to control weed growth on public rights-of-way and in sugar cane, a major Louisiana money crop. These herbicides are often applied as a mixture to increase effectiveness to target vegetation. However, mixed herbicides may combine to form potentially harmful compounds or may enhance the toxic effects of each individual ingredient. One possible nontarget organism that may be affected is the red swamp crawfish *Procambarus clarkii*. Crawfish are an important food source in Louisiana, both to fishing industries and to recreational and subsistence fishermen, as well as an important part of the food chain for many native organisms [1, 2].

Data are available regarding the effects of these herbicides individually on red swamp crawfish, but there is no information available regarding the combined effects of these two herbicides on this species. The following study examined the uptake and excretion of a mixture of these herbicides in the red swamp crawfish,

and addressed the question of whether consumption of exposed crawfish would produce higher or lower health risks than those recorded for single-herbicide exposures.

Materials and Methods

Red swamp crawfish were obtained from the KJEAN Seafood Company of New Orleans, Louisiana. Crawfish were fed oatmeal three times per week and acclimated in holding tanks for 2 weeks. During the bioconcentration and depuration experiments, crawfish were allowed approximately ninety minutes three times weekly to eat oatmeal flakes. Any oatmeal left after feeding concluded was removed to prevent fouling of the water by accelerated bacterial growth.

Bioconcentration Experiment

For the bioconcentration tests, 80 L of aerated and dechlorinated tap water per tank was dosed with one of three concentrations of 2,4-D dimethylamine salt (active ingredient 38.8% 2,4-D) and MSMA (active ingredient 46.33% As), or three 2,4-D/MSMA plus surfactant concentrations. The dosages of the herbicide mixtures were 0.342 mg/L, 0.684 mg/L, and 3.42 mg/L. The highest mixture concentration used was one percent of the 96-hour LC_{50} identified in an earlier series of acute toxicity tests [3]. The lowest mixture concentration was tenfold less than the highest concentration. A control tank of crawfish was also included in the assay [4].

Circulation of the pesticide doses throughout each tank began three days before the addition of crawfish. On a daily basis, 25 L of water was siphoned from the lower portion of each tank and replaced with an equal amount of fresh dechlorinated water and the appropriate dose of the herbicide mixture. Effluent from the tanks was filtered through activated carbon to prevent the herbicides from entering the municipal water supply.

On the first day of the bioconcentration experiment, one hundred seventy-five randomly chosen mixed-sex crawfish were placed in each test tank. Three crawfish were removed from each tank according to the following time schedule: 0 hours, 24 hours, 48 hours, 96 hours, and every fifth day thereafter up until the 47th day, at which point it was decided that the unusually high level of predation within the tanks necessitated an abbreviation of the bioconcentration assay. Ten milliliter water samples were taken from each tank on each sample day, before and after the water were refreshed, to confirm that no more than 20% variation for each herbicides occurred due to the replacement process [4]. Each of the sampled crawfish was dissected into muscle, gill, and hepatopancreas tissues. All samples were frozen until quantification of total arsenic could be performed. Crawfish mortality was recorded daily.

At the end of the bioconcentration period, 3 control crawfish and 3 crawfish from the tank containing the 3.42 ppm mixture concentration were boiled for 20 minutes in one tablespoon of Zatarain's crab and shrimp boil and 3 teaspoons of salt (as recommended in the Zatarain's cooking instructions). Boiled crawfish were dissected as described earlier. These tissues and water samples from the boiling liquid were frozen for later analysis by ICP-MS for total arsenic quantification, as recently described in our laboratory [3].

Depuration

Crawfish remaining from the bioconcentration phase were transferred to fresh dechlorinated water to determine the rate at which they excreted 2,4-D and MSMA from their systems. The total volume of water in each tank was refreshed on a daily basis. Crawfish mortality was recorded daily.

The planned length of the depuration assay was abbreviated due to high predation within the test tanks. Three crawfish were taken from each tank on days 3, 8, 22, and 50 (the final day of the depuration assay). Depuration tissue samples were analyzed using the same methodologies described for the bioaccumulation experiment [3].

Sample Preparation and Analysis

All samples were digested in a CEM MDS-2000 microwave to reduce interference from organic substances and to convert the arsenic to a form that could be analyzed by ICP. For microwave-assisted digestion of water samples, 9 mL of sample and 1 mL of concentrated HNO_3 were initially heated to 160 ± 4°C or 70 psi in 10 minutes. For the second stage, the temperature of the samples was raised to 165 -170°C (or 85 psi) for 10 minutes [3].

Each tissue to be analyzed was defrosted overnight under refrigeration. Aliquots of 0.5 g wet weight of defrosted tissues were microwaved with 9 mL HNO_3; if the weight of the sample was less than 0.5 g ± 0.01g, the actual wet weight was recorded for use in calculating the inorganic arsenic concentration after ICP analysis. Samples were initially heated to 180°C in 12.5 minutes. For the second stage, samples were held at 180°C an additional 9.5 minutes. Each digested sample was diluted by 50% with distilled deionized water before ICP analysis to prevent acid damage to the ICP [3].

Analysis of samples for arsenic concentration was performed using an Agilent Technologies 7500 series inductively coupled plasma mass spectrometer (ICP-MS) with Plasma chromatographic software. Since the molecular weight of arsenic (74.9216) is 46.33 % that of MSMA (161.7), the concentrations of arsenic obtained through ICP analysis was considered to be 46.33% of the actual concentration of MSMA. To correct for the dilution during microwave digestion, ICP results for water samples were multiplied by 1.11. ICP results for tissue samples were multiplied by a correction factor of 0.0526.

Statistical Analysis of Bioconcentration/Depuration Data

Statistical analyses of total arsenic content in tissue samples from the bioconcentration/depuration experiments were performed using three-factor analysis of variance (ANOVA) run by the SAS PROC GLM program [5]. Significant differences were explored using the Newman-Keuls post hoc procedure. Time-specific comparisons were also performed to explore the dose by exposure time effect.

Differences in arsenic concentrations in tissues from the crawfish boil experiment were analyzed for statistical significance using the Student-t test to determine whether boiling removed significant amounts of arsenic from crawfish muscle tissue [6].

Assessment of Human Health Risks

The average doses of arsenic for a person eating crawfish exposed to the herbicide mixtures were calculated to assess the associated health risks. This dose was then compared to the reference dose for arsenic, the dose considered not to affect human health. The equation used for risk assessment was as follows:

$$Dose = \frac{X\ \text{mg accumulated toxicant}}{\text{g crawfish}} \times \frac{Y\ \text{g crawfish consumed}}{\text{day}} \times \frac{1}{Z\ \text{kg}}$$

where, X = the amount of bioconcentrated arsenic in crawfish tissue on the last day of bioconcentration;
Y = the average amount of crawfish flesh ingested per person per day;
Z = 70 kg for adult body weight, and 10 kg for child body weight.

The Louisiana Crawfish Farmers Association reports a rule of thumb for a crawfish boil of 5 lbs of whole crawfish (shell and all) per person and approximately 1 lb of tails (214.75 grams) for every 3 people when cooking crawfish etoufée [7], or 72 grams per person. This quoted average daily intake of tail meat per person was used as the high end of the range for the amount of crawfish consumed. The low end of the range for this variable was drawn from the conventionally accepted value of 33 grams per person of seafood (fish or shellfish) consumed daily. The risk or margin of exposure (MOE), for each treatment was calculated by dividing each treatment's exposure dose by the accepted reference dose for arsenic.

The cancer risk for a lifetime of ingestion of crawfish exposed to a 3.42 ppm mixture of 2,4-D and MSMA, with and without surfactant, was determined using the following equation:

$$\text{Cancer Risk} = \text{Dose} \times \text{Oral Slope Factor}$$

in which Dose equals the exposure dose calculated in the systemic (non-cancer related) health risk evaluation, and the oral slope factor used was the EPA's estimate of 1.5 $(mg/kg/day)^{-1}$.

Results

Bioconcentration Phase

Statistical analyses of inorganic arsenic content in tissue samples from the bioconcentration/depuration experiment yielded the results listed in Table 1.

Table 1: Statistical analysis of bioconcentration / depuration data

Source	*Mean square*	*F*	*p*
Tissue (Ti)	49.12	133.10	< 0.0001
Dose (D)	14.48	39.24	< 0.0001
Ti x D	2.46	6.68	< 0.0001
Time (T)	1.29	3.51	< 0.0001
Ti x T	0.84	2.29	0.0009
D x T	0.62	1.68	0.0278
Ti x D x T	0.84	2.26	0.0001
Standard Error	0.37		

The average inorganic arsenic concentrations detected in each tissue over total time of the bioaccumulation/depuration experiment are listed in Table 2. Calculated across all of the tissues over the total time of the assay, the arsenic tissue concentrations observed were 0.59 ppm for the control, 1.24 ppm for the 3.42 treatment, and 1.12 ppm for the 3.42 ppm + surfactant treatment. Arsenic concentrations measured in control tissues were significantly lower than those measured in the test groups ($p < 0.01$). The presence of surfactant was not observed to cause a significant difference in arsenic concentrations measured across tissues over the total time of the bioconcentration experiment.

Table 2: Average inorganic arsenic concentrations detected in each tissue over total time

Tissue	*Control*	*3.42 ppm As exposure*	*3.42 ppm As + surfactant exposure*
Hepatopancreas	1.05 ppm	2.24 ppm	1.88 ppm
Gill	0.38 ppm	0.75 ppm	0.93 ppm
Muscle	0.32 ppm	0.73 ppm	0.55 ppm

Table 3 lists the dose by tissue interactions of arsenic in sampled tissues. Hepatopancreas bioconcentrated the highest amounts of arsenic of the three tissues sampled, at 1.88 ppm and 2.24 ppm total arsenic for tissues exposed to the herbicide mixture with and without surfactant respectively. These concentrations were lower than those found by Abdelghani et al. [1] of 3.7 ppm arsenic at the long-term exposure to an MSMA-only concentration of 1.1 mg/L. A significantly greater amount of arsenic was bioconcentrated in the absence of surfactant ($p < 0.01$).

Table 3: Dose by tissue interactions of arsenic concentrations

Group	*Hepatopancreas*	*Gill*	*Muscle*
Control vs. 3.42 ppm	Significant difference ($p < 0.01$)	Significant difference ($p < 0.01$)	Significant difference ($p < 0.01$)
Control vs. 3.42 ppm + surfactant	Significant difference ($p < 0.01$)	Significant difference ($p < 0.01$)	No significant difference
3.42 ppm vs. 3.42 ppm + surfactant	Significant difference ($p < 0.01$)	No significant difference	No significant difference

Gill tissue was observed to bioconcentrate significant amounts of arsenic ($p < 0.01$) in both treatments (0.75 ppm without surfactant and 0.93 ppm with surfactant), but its uptake of arsenic was not significantly affected by the presence of surfactant. Muscle tissue displayed no significant difference in long-term arsenic concentration between the control and the treatment with surfactant, with average concentrations over time of 0.32 ppm and 0.55 ppm, respectively. Surfactant was not observed to significantly affect the accumulation of arsenic in muscle tissue, with concentrations averaging at 0.55 ppm and 0.73 ppm, in the treatments with and without surfactant respectively. Muscle tissue sampled from the treatment alone did, however, contain significantly higher concentrations of arsenic ($p < 0.01$) than those sampled from the control group, at overall concentrations of 0.32 ppm and 0.73 ppm, respectively.

Depuration Phase

Abdelghani et al [1] reported that after long-term exposure to MSMA, gill tissue displayed the greatest total arsenic loss of the three tissues under study (71%-78%). In contrast, gill tissue after long-term exposure to the 50:50 2,4-D/MSMA mixture displayed a total arsenic loss of approximately 41% (from 0.68 ppm to 0.40 ppm). Gill tissue after long-term exposure to the mixture with surfactant added displayed a total arsenic loss of approximately 46% (from 0.72 ppm to 0.39 ppm). Hepatopancreas tissue samples lost 63% of their arsenic content (from 3.21 ppm to 1.18 pm) in the absence of surfactant and 67% of their arsenic content (from 3.26 ppm to 1.08 ppm) in the presence of surfactant. Muscle tissues lost 50% of arsenic content (from 1.15 ppm to 0.58 ppm) in the absence of surfactant and 29% (from 0.68 ppm to 0.48 ppm) in the presence of surfactant. The order of loss based on percent of total arsenic depurated from the tissues in the absence of surfactant is as follows:

hepatopancreas > muscle > gills

and the order of loss based on percent of total arsenic depurated from the tissues in the presence of surfactant is as follows:

hepatopancreas > gills > muscle

This is in contrast to Abdelghani's findings from a bioconcentration system with MSMA exposure alone:

gills > hepatopancreas > muscle

Average arsenic concentrations from the final day of bioconcentration to the final day of depuration are listed in Tables 4 and 5. Statistical comparisons of the highest herbicide mixture treatments with and without surfactant showed a significant loss of arsenic across tissues from both treatments during the first 24 hours of depuration. Total arsenic concentration in tissues decreased from 1.68 ppm to 1.10 ppm in the mixture treatment and from 1.56 ppm to 0.97 ppm in the treatment with surfactant (both at $p < 0.05$). Significant differences in arsenic concentrations were also found between the last day of bioconcentration and the last day of depuration, with tissue arsenic decreasing from 1.68 ppm to 0.72 ppm in the mixture treatment and from 1.56 ppm to 0.65 ppm in the treatment with surfactant (both at $p < 0.01$).

Table 4: Tissue arsenic concentrations from final bioconcentration day to final depuration day, 3.42 ppm treatment

Time	*Hepatopancreas*	*Gill*	*Muscle*	*Average*
Day 0	3.21	0.68	1.15	1.68
Day 3	1.69	0.65	0.95	1.10
Day 8	1.40	0.45	0.64	0.83
Day 22	1.32	1.27	0.59	1.06
Day 50	1.18	0.40	0.58	0.72

Table 5: Total arsenic concentrations from final bioconcentration day to final depuration day, 3.42 ppm + surfactant treatment

Time	*Hepatopancreas*	*Gill*	*Muscle*	*Average*
Day 0	3.26	0.72	0.68	1.56
Day 3	1.86	0.58	0.47	0.97
Day 8	1.60	0.55	0.64	0.93
Day 22	1.61	1.38	0.53	0.84
Day 50	1.08	0.39	0.48	0.65

Total Arsenic Content in Boiled Crawfish

Table 6 lists the average arsenic concentrations in tissues used to examine the effects of cooking on arsenic content. Boiled muscle tissue from exposed crawfish contained a significantly higher amount of total arsenic than boiled muscle tissue from control crawfish ($p < 0.003$), at 0.76 ppm and 0.29 ppm respectively. Boiled treated muscle tissue did not, however, contain a significantly different concentration of arsenic than uncooked treated tissue ($p \leq 0.310$), at 0.76 ppm and 1.15 ppm, respectively. The only significant difference in arsenic content between the uncooked treatment tissues and the boiled treatment tissues was found in the hepatopancreas tissue concentrations ($p < 0.006$), with total arsenic concentrations of 3.21 ppm and 0.58 ppm, respectively.

Table 6: Total arsenic content in boiled crawfish (in ppm)

Tissue	*Boiled control*	*Boiled 3.42 ppm*	*Uncooked 3.42 ppm*
Hepatopancreas	0.58	1.12	3.21
Gills	0.30	0.61	0.68
Muscle	0.29	0.76	1.15

The gill tissues sampled for this assay were not observed to have bioconcentrated a significant amount of arsenic 0.5L ($p < 0.605$), which is contrary to findings from the bioaccumulation assay. This is probably due to random differences in the uptake of arsenic from crawfish to crawfish and to the fact that the sampling pool for the boiling assay was a relatively small one.

Assessment of Human Health Risks

Human health risks were calculated using arsenic tissue concentrations from the second to last day of bioconcentration sampling. Use of the risk assessment equation described in section 5.7 yielded the following results:

1. Herbicide mixture dose = 2.9×10^{-4} to 6.4×10^{-4} mg/kg/day (an MOE of 1 to 2.1)

2. Herbicide mixture with surfactant dose = 2.3×10^{-4} to 5×10^{-4} mg/kg/day (an MOE of 0.77 to 1.6)

The averages of these doses are higher than the accepted reference dose for inorganic arsenic of 3×10^{-4} mg/kg/day. Both herbicide treatments yielded margins of exposure that were approximately twice the acceptable level of one.

Estimated arsenic-related cancer risks for ingestion of crawfish that underwent long-term exposure to the 2,4-D/MSMA mixture were quantified at 4 - 10 extra tumors in a population of 10,000 over a lifetime consumption of crawfish exposed to the herbicide mixture, or 4 - 8 extra tumors in a population of 10,000 over a lifetime of consumption of crawfish exposed to the mixture plus surfactant. These cancer risks averaged to approximately 7 extra tumors in a population of 10,000 resulting from a lifetime consumption of crawfish exposed to the herbicide mixture and 6 extra tumors in a population of 10,000 resulting from a lifetime consumption of crawfish exposed to the herbicide mixture with surfactant. This method computes the 95% upper bound for the risk rather than the average risk, which results in there being a very good chance that the risk is actually lower. These calculated cancer risks are considered to be good within one order of magnitude; in other words, 10^{-4} may in actuality be 10^{-5}

Discussion

Bioconcentration

Crawfish have an “open” circulatory system with arteries that eventually terminate after leaving the heart, allowing circulatory fluid, or hemolymph, to bathe the internal organs [8]. All dissected tissues were therefore in constant contact with the herbicide mixture introduced into the crawfish hemolymph through absorption from the gills [9]. Arsenic binds to the sulfhydryl groups of hemocytes in the hemolymph as it passes through the gills, which are directly exposed to the contaminate medium.

The arsenic-laden hemolymph then moves through the hepatopancreas, where the bound metals are concentrated and sequestered to minimize toxicity [1, 10]. The crawfish hepatopancreas serves in a variety of physiological processes, including digestion, absorption and storage of digested foods, detoxification, and storage of heavy metals [10 - 12]. In vertebrate hepatopancreas and liver tissue, arsenic induces production of metallothioneins, a class of low molecular weight proteins which bind metals such as arsenic, thereby rendering them unavailable to cause cellular damage [13, 14]. In invertebrates, arsenic-induced metallothioneins

do not actually bind arsenic [15]; instead, the primary method of arsenic sequestration in invertebrate hepatopancreas appears to be the formation of intracellular vacuoles [11, 16]. Approximately 27% of the arsenic also binds to the lipid fraction of the hepatopancreas [1]. This multifaceted ability to concentrate and sequester arsenic explains why the hepatopancreas was not only observed to accumulate higher amounts of total arsenic than gill or muscle tissue but was also found to contain the highest concentrations of arsenic in control tissues.

The significantly lower arsenic concentrations found in hepatopancreas samples exposed to the mixture with surfactant added may be due to the ability of surfactant to adsorb metal ions into precipitates of metal-surfactant. Surfactants added to a solution can render hydrophilic mineral surfaces hydrophobic through the formation of neutral metal-surfactant molecules [17]. This adsorptive activity led to arsenate removals of over 90% in the remediation studies of Lazaridis et al [18]. In comparison, the 1.88 ppm average total arsenic concentrated in the hepatopancreas exposed to surfactant in the bioconcentration experiment was 84% less than the 2.24 ppm average total arsenic concentrated in the hepatopancreas samples in the absence of surfactant.

The gills are in direct contact with water and present a relatively large permeable surface for exchange of water-borne chemicals [8, 19]. Oxygen consumption in gill tissue decreases in the presence of heavy metals. Respiratory stress may therefore affect the overall metabolic processes involved in the concentration and elimination of arsenic [8]. Gill tissue bioconcentrated significant amounts of arsenic, reaching an arsenic plateau around day 30 of the bioconcentration assay. The rest of the arsenic entering the gills would have been transported away as gill tissues were flushed by hemolymph [1].

Crawfish abdominal muscle has consistently been found in literature to contain the lowest concentration of metals of all sampled crawfish tissues, a finding that was paralleled by the results from the bioconcentration assay. For example, Jorhem et al [20] reported a total arsenic bioconcentration of 0.18 g/g (ppm) in muscle tissue, 4.5 times less arsenic than the concentration they found in hepatopancreas (0.81 g/g (ppm)). In the 2,4-D/MSMA mixture bioconcentration assay, muscle bioconcentrated 3.1 times less arsenic than hepatopancreas (0.73 ppm versus 2.24 ppm). These findings are important in risk assessments to human health, since muscle tissue is the most often consumed portion of the crawfish. Composed of 81% water, crawfish abdominal muscle is likely to have fewer arsenic binding sites than the other tissues studied in this experiment [1]. The adsorption of arsenic by the surfactant may also have prevented a significant portion of arsenic from concentrating in muscle tissue.

2,4-D can affect enzymatic activity during long-term exposure (Neskovic et al, 1996). It is therefore possible that the presence of 2,4-D in the herbicide mixture altered the ability of hepatopancreas to depurate stored products of MSMA. 2,4- D may also have decreased the ability of gill tissue to bioconcentrate MSMA, thereby reducing the amount of MSMA products available for depuration.

Effects of Cooking on Arsenic Concentration

The amount of arsenic bioconcentrated into muscle tissue was not significantly affected by boiling. This may be due to tight binding of arsenic at available sites within muscle tissue [1]. Muscle proteins are denatured during cooking, but this does not seem to significantly affect the proteins involved in arsenic sequestration.

The significant difference in arsenic content between the uncooked exposed hepatopancreas and the boiled exposed hepatopancreas may have been due to the loss of hepatopancreatic lipids to the boiling medium. The hepatopancreas showed a dramatic alteration in size and consistency after boiling. The structural dissolution of this tissue would release arsenic bound to hemocytes and lipids and sequestered within intracellular vacuoles.

Human Health Risk Assessment

Risk assessments for consumption of tissues exposed to both herbicide treatments yielded margins of exposure that were approximately twice the acceptable level. An unknown fraction of the total arsenic findings would actually be present in relatively nontoxic organic forms such as arsenobetaine [21]; therefore the risk assessments performed from this experiment may be misleading. Estimated arsenic-related cancer risks for ingestion of these crawfish yielded an average risk of 6 and 7 tumors in a population of 10,000; the accepted reference dose for arsenic yields a cancer risk of 4.5, or 5 extra tumors in a population of 10,000 over a lifetime's consumption of exposed crawfish.

One element not examined by this series of assays concerns the forms of arsenic that the bioaccumulated element might have been stored in. Different species of arsenic have different levels of toxicity [21, 22]. For example, arsenobetaine, which is the major converted form of arsenic found in various marine animals, has been shown to be relatively non-toxic [21]. It would be valuable to determine how large a fraction of the arsenic bioconcentrated in this study was actually stored in this and other relatively nontoxic forms. Such information would give a clearer understanding of the actual risk involved in consuming these crawfish.

References

1. Abdelghani, A. A.; Mason, J. W.; Anderson, A. C.; Englande, A. J.: Bioconcentration of MSMA in crayfish (*Procambarus* sp). In Hemphill, D. D., Ed. *Trace Substances in Environmental Health* -X. University of Missouri, Columbia. **1976.**
2. Abdelghani, A. A.; Tchounwou, P. B.; Anderson, A. C.; Sujono, H.; Heyer, L. R.; Monkiedje, A.:

Toxicity evaluation of single and chemical mixtures of roundup, Garlon-3A, 2,4-D and Syndets Surfactant to channel catfish (*Ictalurus punctatus*), bluegill sunfish (*Lepomis microchirus*), and crawfish (*Procambarus* spp.). *Environ. Toxicol. Water Quality* **1997,** *12(3)*, 237 - 243.

3. Green, R. M.; Abdelghani, A. A.: Toxicity of a mixture of 2,4-Dichlorophenoxyacetic acid and monododium methanecarbonate to the red swamp crawfish, *Procambarus clarkia. Int. J. Environ. Res. Public Health,* **2004***, 1,* 35-38.
4. United States Environmental Protection Agency. OPPTS 850.1730: Fish BCF. "Public Draft". *Ecological Effects Test Guidelines* EPA 712-C-96-129. **1996.**
5. SAS PROC GLM. General Linear Model software, version 8.0. **1999.**
6. GraphPad.com. 2001. GraphPad Student-t test. [http://www.graphpad.com/calculators/ttest1.cfm]
7. Deshotel, R. Personal communication. **2003.**
8. Martin, G. G.; Hose, J. E.: Vascular elements and blood (hemolymph). In Harrison, F. W. and A. G. Humes, Eds.) *Microscopic Anatomy of Invertebrates, Volume 10: Decapod Crustacea*. New York: Wiley-Liss, Inc. **1992**.
9. Hebel, D. K.; Jones, M. B.; Depledge, M. H.: Responses of Crustaceans to Contaminant exposure: a holistic approach. *Estuarine, Coastal, and Shelf Science* **1997**, *44*, 177-184.
10. Anderson, M. B.; Reddy, P.; Preslan, J. E.; Fingerman, M.; Bollinger, J.; Jolibois L.; Maheshwarudu, G.; George, W. J.: Metal accumulation in crayfish, *Procambarus clarkii*, exposed to a petroleum-contaminated bayou in Louisiana. *Ecotoxicol. Environ. Safety* **1997**, *37*, 267-272.
11. Coombs, T. L.; George, S. C.: Mechanisms of immobilization and detoxification of metals in marine organisms. In McLusky, D.S. and A.J. Berry, Eds. *Physiology and Behaviour of Marine Organisms*. London: Pergamon Press. **1978**.
12. Icely, J. D.; Nott J. A.: Digestion and absorption: Digestive systems and associated organisms. In Harrison, F. W. and Humes, A. G. Eds. *Microscopic Anatomy of Invertebrates, Volume 10: Decapod Crustacea*. New York: Wiley-Liss, Inc. **1992**.
13. Del Razo, L. M.; Quintanilla-Vega, B.; Brambila-Colombres, E.; Calderon-Aranda, E. S.; Manno, M.; Albores, A.: Stress proteins induced by arsenic. *Toxicol. Appl. Pharmacol.* **2001**, *177,* 132-148.
14. Mouneyrac, C.; Amiard-Triquet, C.; Amiard, J. C.; Rainbow, P. S.: Comparison of metallothionein concentrations and tissue distribution of trace metals in crabs (*Pachygrapsus marmoratus*) from a metal-rich estuary, in and out of the reproductive season. *Comparative Biochem. Physiol. Part C,* **2001**, *129*, 193-209.
15. Mourgaud, Y.; Martinez, E.; Geffard, A.; Andral, B.; Stanisiere, J. Y.; Amiard, J. C.: Metallothionein concentration in the mussel *Mytilus galloprovincialis* as a biomarker of response to metal contamination: validation in the field. *Biomarkers* **2002,** *7(6)*, 479-490.
16. Roldan, B. M.; Shivers, R. R.: The uptake and storage of iron and lead in cells of the crayfish (*Orconectes propinquus*) hepatopancreas and antennal gland. *Comparative Biochem. Physiol.* **1987,** *86C (1)*, 201-214.
17. Fuerstenal, M. C.; Han, K. N.: Metal-surfactant precipitation and adsorption in froth flotation. *J. of Colloid and Interface Sc.* **2002**, *256*, 175-182.
18. Lazaridis, N. K.; Hoursemanoglou, A; Matis, K. A.: Flotation of metal-loaded clay anion exchangers. Part II: the case of arsenates. *Chemosphere* **2002**, *47*, 319-324.
19. Taylor, H. H.; Taylor, E. W.: Gills and lungs: the exchange of gases and ions. In Harrison, F.W. and Humes, A. G. Eds. *Microscopic Anatomy of Invertebrates, Volume 10: Decapod Crustacea*. New York: Wiley-Liss, Inc. **1992**.
20. Jorhem L.; Engman, J.; Sundström B.; Thim, A. M.: Trace elements in crayfish: regional differences and changes induced by cooking. *Archives of Environ. Contam. Toxicol.* **1994**, *26*, 137-142.
21. Velez, D.; Ybáñes, N.; Montoro, R.: Percentages of total arsenic represented by arsenobetaine levels of manufactured seafood products. *J. of Agric. Food Chem.* **1995**, *43*, 1289 - 1294.
22. Tchounwou, P. B.; Wilson, B. A.; Abdelghani, A. A.; Ishaque, A. B.; Patlolla, A. K.: Differential cytotocity and gene expression in human liver carcinoma cells exposed to arsenic trioxide and monosodium acid methane arsonate. *Int. J. Mol. Sci.*, **2002**, *3*, 1117-1132
23. Tchounwou, P. B.; Patlolla, A. K.; Ceneteno J. A.: Carcinogenic and systematic health effects associates with arsenic exposure – a critical review. *Toxicol. Pathol*., **2003**, *31*, 575-588