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Differential Effects of Arsenite and Arsenate to *Drosophila melanogaster* in a Combined Adult/Developmental Toxicity Assay

S. H. Goldstein[1] and H. Babich[1,2]

[1]Department of Biological Sciences, Stern College, Yeshiva University, 245 Lexington Avenue, New York, New York 10016 and [2]Laboratory Animal Research Center, The Rockefeller University, 1230 York Avenue, New York, New York 10021

Current concern of the environmental consequences of chemical wastes in soils has led to the development of microbial (Babich and Stotzky 1983; Babich et al. 1983), plant (Thomas and Cline 1985; Wang 1987), and, to a lesser extent, animal (e.g., earthworm) (Thomas et al. 1986) bioassays for terrestrial ecosystems. This paper evaluated a Drosophila assay that yields data both on acute toxicity to adults and on developmental toxicity to offspring and which is applicable for screening extracts from soils contaminated with chemical wastes.

Acute toxicity assays with Drosophila have been used to evaluate the relative potencies of chemicals. For example, Williams et al. (1982) applied an acute toxicity assay with Drosophila for establishing structure activity relationships (SARs) for a series of di- and trivalent inorganic cationic metals. An earlier report from that laboratory (Jacobson et al. 1981) evaluated a developmental exposure assay to assess the yield of adult flies that emerged when Drosophila spent their entire life cycle (embryo, larval, pupal, and adult stages) in cadmium amended medium. The acute toxicity to adults and the developmental exposure bioassays were designed to be performed as separate tests. This paper combined these two tests into a single bioassay, using arsenic compounds as the test agents. Arsenite and arsenate were selected to evaluate the sensitivity of this combined assay in distinguishing between the toxicities of closely related chemicals. Furthermore, arsenic compounds are important environmental contaminants. Global anthropogenic input of arsenics into soils has been estimated at 5.2 to 11.2 x 10^7 kg/yr (Nriagu and Pacyna 1988).

Send reprint requests to H. Babich at Stern College

MATERIALS AND METHODS

Wild type Oregon-R *Drosophila melanogaster* were maintained in plastic vials (50 cm^3) containing Instant *Drosophila* Medium (Carolina Biological) and housed in a humidified incubator at 20 C. Cultures were transferred every two-three weeks.

Ten male and ten female flies were transferred to vials containing medium unamended and amended with 0.1, 0.25, 0.5, 0.75, 1.0, 1.25, 1.5, and 2.0 mM arsenite, as $NaAsO_2$, or arsenate, as Na_2HAsO_4. Cultures were examined the following day for flies that may have died from the trauma of transferring; such flies were not incorporated into the acute toxicity data. The cultures were maintained for 7 days and the surviving flies were scored. Percent survival data were calculated and acute toxicity curves were constructed. Viable, adult flies were then discarded and the vials were maintained for another 2 weeks, during which time the eggs deposited into the media, both control and arsenic amended, progressed through larval and pupal stages, and then to adults. The length of the life cycle of *Drosophila* maintained at 20 C is about 15 days (Demerec and Kaufmann 1965), thus, the newly emerging adult flies were from eggs deposited by the parental flies, rather than by the F_1 generation. The number of flies was scored and developmental toxicity curves were constructed.

Two replicates were used for each concentration of toxicant and the experiments were performed three times. The data points on the toxicity curves are presented as the arithmetic mean ± standard error of the mean. Acute toxicity LC_{50} values and midpoint developmental toxicity values were determined by linear regression analysis.

RESULTS AND DISCUSSION

In the acute toxicity assay the number of surviving flies was determined after 4 and 7 days of exposure. Differential toxicities between arsenite and arsenate were more pronounced after 7, rather than 4, days of exposure. After 4 days of exposure a reduction in survival of about 10% was noted for flies exposed to 0.25 to 0.75 mM arsenite or arsenate and of about 15 and 25% for flies exposed to 1.0 mM arsenate and arsenite, respectively. Complete toxicity curves were generated after 7 days of exposure (Figure 1), with the 7 day LC_{50} values being 0.54 mM arsenite and 0.79 mM arsenate. Similar studies with white-eyed mutants also noted the greater toxicity of arsenite than of arsenate (data not presented).

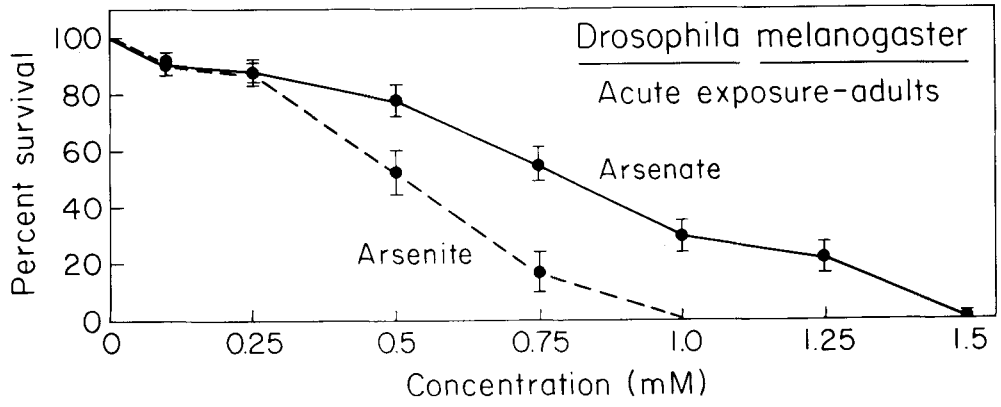

Figure 1. Survival of Drosophila melanogaster after 7 days of exposure to medium amended with varied concentrations of arsenite or arsenate. The data are presented as the arithmetic means ± SEM.

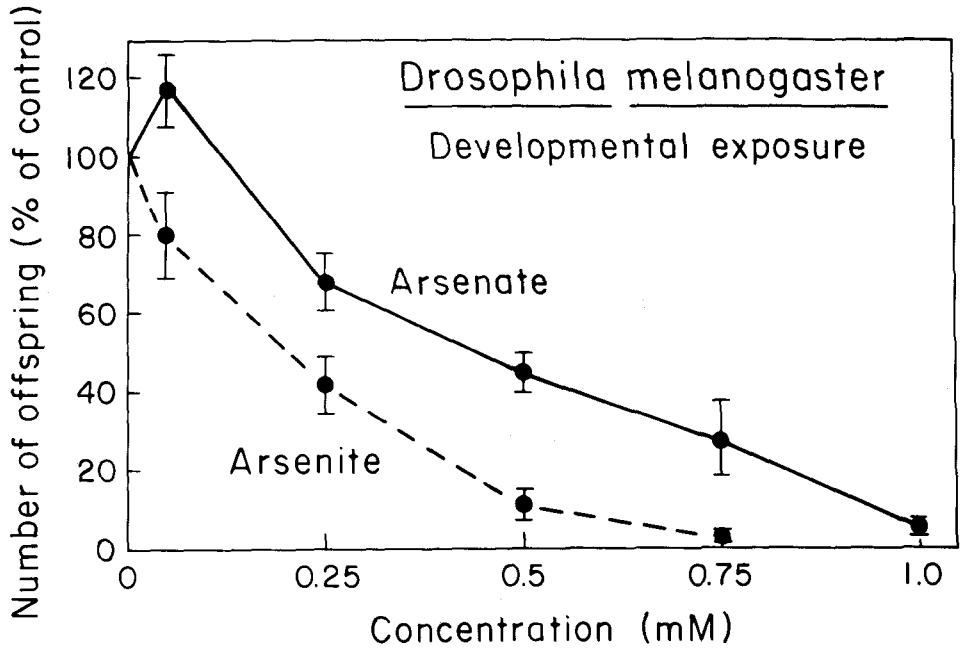

Figure 2. Reduction in the yield of Drosophila melanogaster by various concentrations of arsenite and arsenate. The data are presented as the arithmetic mean ± SEM.

In the developmental exposure assay, the number of adult flies emerging from the control and arsenic amended media was determined 3 weeks after the initial introduction of the 10 parental male and female flies into the vials. Arsenite was more toxic than arsenate (Figure 2), with the midpoint toxicity values for the developmental exposure assay being 0.21 mM arsenite and 0.45 mM arsenate.

Reduction in the yield of adult flies in the developmental exposure was, apparently, not merely a reflection of the acute toxicities of the arsenics to the parental flies during their 7 day exposure. Thus, 0.25 mM arsenite or arsenate was not significantly toxic to the parental flies (Figure 1), but in the developmental exposure assay these concentrations reduced the yield of the flies by about 60 and 40%, respectively (Figure 1).

Arsenite was more toxic than arsenate, both to the survival of adult flies (Figure 1) and to the developmental processes from egg to young adult (Figure 2). This greater toxicity of arsenite than of arsenate was consistent with studies using bacteria (Anderson and Abdelghani 1980; Liu 1981), fish (Babich et al. 1986) and mammalian (Borenfreund and Puerner 1986) cells in culture, and laboratory animals (Franke and Moxon 1936; Maitani et al. 1987). The differential toxicities of these arsenics have been attributed to their distinct modes of action. Arsenite is an enzyme inhibitor, specifically interacting with sulfhydryl groups; arsenate is a structural analog of phosphate and, as such, is an uncoupler of oxidative phosphorylation (Leonard and Lauwerys 1980; Coddington 1986).

To be applicable for risk assessment, toxicity tests for terrestrial environments must encompass the multiple levels of ecosystem complexity (Burton and Stemmer 1988). Terrestrial bioassays have focused primarily on microbial and phytotoxicity assays, with only some attention directed to the animal component (Porcella 1983). The Drosophila bioassay described herein can be performed with water-extractable chemical components of waste site soils to provide two distinct aspects of ecotoxicity data on an animal component of terrestrial ecosystems.

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