Bull. Environ. Contam. Toxicol. (1990) 45:876–882

Environmental Contamination and Toxicology

Pesticide Perturbation of Sperm Cell Function

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Pesticides from agricultural, manufacturing and recreational sites and oil spills as well as toxicants dispersed as aerial sprays ultimately find their way into the marine ecosystem. In the coastal waters they can impinge adversely on the reproductive processes of marine organisms and their predators. If the toxicants were to affect the males as well as the females, the sperm cell could well serve as a readily accessible index of the degree of pollution. The spermatozoon, though neither a nerve nor a muscle cell, nevertheless exhibits some of the salient attributes of each encompassing a broad range of physiological specializations. The highly differentiated cell possesses a condensed, metabolically inactive nucleus and a relatively restricted cytoplasmic compartment (Baccetti 1985) and is characterized by an exceptionally large surface/volume (s/v) ratio. Under these circumstances, cell functions mediated by activation of factors located in the cell membrane may be particularly vulnerable. Whereas the s/v ratio of a "typical" cell may approach unity, that of a sperm cell may exceed 50:1. This is especially relevant in studies on the effects of pesticides, many of which, though quite insoluble in aqueous media, are highly lipophilic, which favors ready permeation of plasma membranes (Hoskin and Rosenberg 1965). About 23% of the bull sperm flagellum consists of lipid (Zittle and O'Dell 1941) and phospholipids comprise about 70% of the total lipid content of isolated and purified sperm cell plasma membranes (Nikolopoulau et al 1985). Compared to mammalian sperm cells, those of marine invertebrates possess thinner, relatively longer flagella giving them as much as a 100:1 s/v ratio. A broad spectrum of pharmacological substances interact with specific receptors that regulate sperm cell motile behavior. For example, neurohumoral factors play a significant role (Nelson 1972, Bavister et al 1979; Nelson and Cariello 1989), as well as ion channel blocking agents (Young and Nelson 1974), and cyclic nucleotide modulating agents (Casillas and Hoskins 1971; Garbers et al 1971; Schoff and Lardy 1987). Pesticide disruption of reproductive processes may, in part, be due to adverse effects on sperm cell function. The present study was undertaken to monitor motility responses of sperm cells to pesticides. The data will provide baseline motility values essential for the design of experiments to determine underlying control mechanisms specifically affected by the pesticides.

MATERIALS AND METHODS

Freshly caught specimens of the American sea urchin, Arbacia punctulata, were maintained in running sea water aquaria at the Marine Biological Laboratory, Woods Hole, Massachusetts, during July and August, 1989. Low voltage electrical stimulus to the test (shell of the echinoderm) enables sexing, and intraperitoneal injection of the males with 1 mL of 0.5 M KCl induced spawning of the mature sperm cells. The spermatozoa were collected in a coherent stream under chilled artificial sea water (ASW), salinity, 34.6 g/kg, buffered at pH 7.4. One liter of ASW contains 27.42 g NaCl, 0.67 g KCl, 1.36 g $CaCl_2.2\ H_2O$, 9.90 g $MgCl_2.6\ H_2O$ and 0.18 g $NaHCO_3$. A 100 μL aliquot of the concentrated semen diluted to 25 mL in ASW provided enough material for each set of experimental runs. An optical density (OD) of 0.5 - 0.7 at wavelength 540 nm in a Bausch and Lomb Spectronic 340 spectrophotometer was equal to a sperm count of 10^6 to 10^7 cells/mL. Six cuvettes were used for each experiment: 0.3 mL of a given concentration of the test pesticide was pipetted into cuvettes #1 - #3; cuvettes #4 and #5, controls, received 0.3 mL ASW; and, to fix and immobilize the cells, 0.3 mL of 10% formaldehyde was added to cuvette #6. A 2.7 mL aliquot of the diluted sperm suspension was added to each of the cuvettes and the cells were dispersed by vortical swirling and the OD at Time 0 were recorded. The cells were oriented in the horizontal rotor of a table-top centrifuge at 120 x g for 4 min. The ODs at Time 4 min were recorded. The formaldehyde-immobilized cells are displaced very little at this RCF but any change in their OD serves as a correction factor for the presence of non-motile cells in the samples (Nelson 1972). The sperm cells were redispersed, the ODs again recorded preparatory to a second and then a third orientation spin. These operations required about 10 min for each succeeding run and so the incubation intervals, as indicated on the graphs, occurred at 5, 15 and 25 min. All experiments were conducted at room temperature (20 - 23° C) and replicated 6 - 12 times.

Three categories of pesticides were assayed: an organophosphate, paraoxon; organochlorines, dieldrin, lindane and mirex; organometals, tributyltin acetate and ethylmercuric chloride and the inorganic salt, $HgCl_2$. The pesticides, of 95-99% purity, were purchased from ChemService, Inc., West Chester, Pennsylvania. They were dissolved or colloidally dispersed in dimethyl sulfoxide which was obtained along with other reagent grade chemicals from Sigma Chem. Co., St. Louis, Missouri.

RESULTS AND DISCUSSION

Spermatozoa appear to be particularly compelling models for investigating the cellular basis of toxicity. The pesticides exerted differential effects on the sperm cells providing valuable insights into mechanisms that may underlie deficits in male reproductive function.

The abscissae show the incubation time in minutes and the ordinates indicate the relative rate of motile progression of the treated

cells normalized to that of the controls at 100% (Figures 1 - 7). The anticholinesterase, paraoxon, an oxidized but active metabolite of the agricultural insecticide parathion, elicits a biphasic response. Initially stimulating spermatozoan movement to 175% of that of the controls the sperm speed then reverted, in the 0.74 to 14.8 n*M* range, toward the control level; paraoxon at 69.8 n*M* caused only a slight initial increase which then dropped to about 50% of the controls during the 15 and 25 min incubation periods (Fig 1). The biphasic response may be ascribed to the sparing action of intracellularly synthesized acetylcholine by other anticholinesterases, e.g., eserine, neostigmine, diisopropylfluorophosphate (Nelson 1972).

Of the organochlorines tested, the cyclodiene, dieldrin (Shaikland 1982), evoked a swimming response somewhat similar in form to that caused by paraoxon. Stimulation in the case of dieldrin (Fig 2), however, was limited to only about 20% above the control rate in the 1 - 30 micromolar range while at 92 μM the initial response was an inhibition of about 60%. Lindane, the γ-isomer of hexachlorocyclohexane, induced primarily inhibitory responses although at the lowest concentration, 130 n*M*, this toxicant lacked any apparent effect on motility (Fig 3). In sharp contrast, mirex was markedly stimulatory. This insecticide which may be oxidized to chlordecone, another extremely persistent chlorinated hydrocarbon, is concentrated several thousandfold in the food chain (Waters et al 1977). After prolonged exposure, mirex doubles the rate of sperm progression at an optimum concentration of 100 nanomolar (Fig 4). Below that concentration, the motility did not differ significantly from the controls while at higher doses, the stimulatory effect was less pronounced. These responses may be related to mirex's known stimulatory effects on the vertebrate CNS. Mirex also causes testicular atrophy in humans (Eroshenko 1981).

In general, the metallic compounds tended to exert primarily depressant effects on the sperm cells. Organotin compounds exist in a multiplicity of forms, both with respect to the alkyl substituent and to the valence state of the metal, each of which may affect any of a diversity of cellular processes (Snoeij et al 1987). Tributyltin acetate, the more potent of the compounds tested, was effective in a range extending from 21 picomolar, causing a progressive decrease in motile rate with time of exposure (Fig 5). It is not clear at present why an apparently anomalous increase in motile rate should occur at 210 p*M* when both higher and lower concentrations cause decreases in the motile rate.

Organomercurials reportedly have higher affinities than do their inorganic analogs for marine organisms (Fowler et al 1978). Ethylmercuric chloride appears to be more toxic at lower than at higher concentrations (Fig 6). This may reflect diminished solubility with increasing concentration. A 90% inhibition occurs at 150 n*M*, while at 1.1 μM the organometal has a slight stimulatory effect and at 5.5 μM the motile rate remains at the same level as

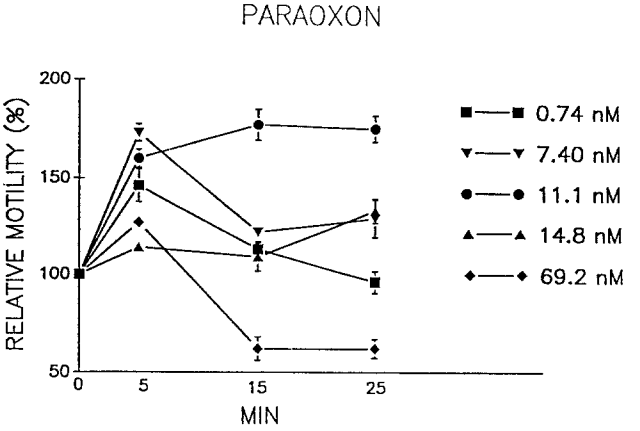
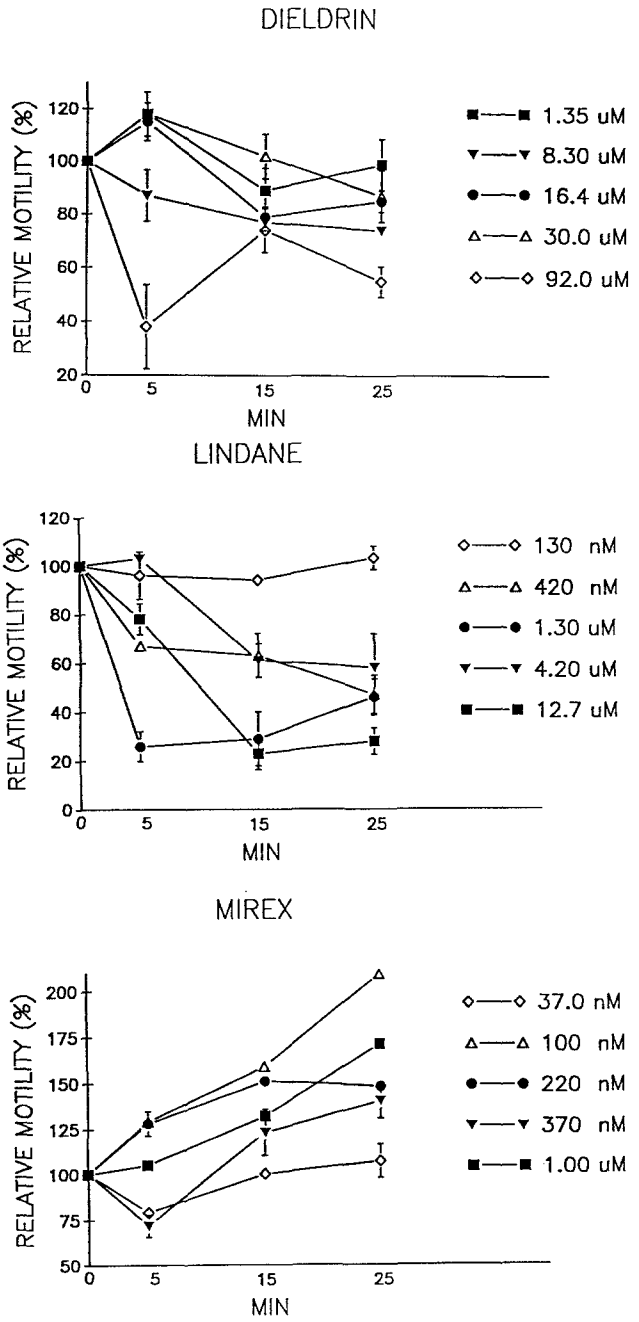


Figure 1. Effects of paraoxon on the rate of motile progression of the spermatozoa of Arbacia punctulata in artificial sea water.

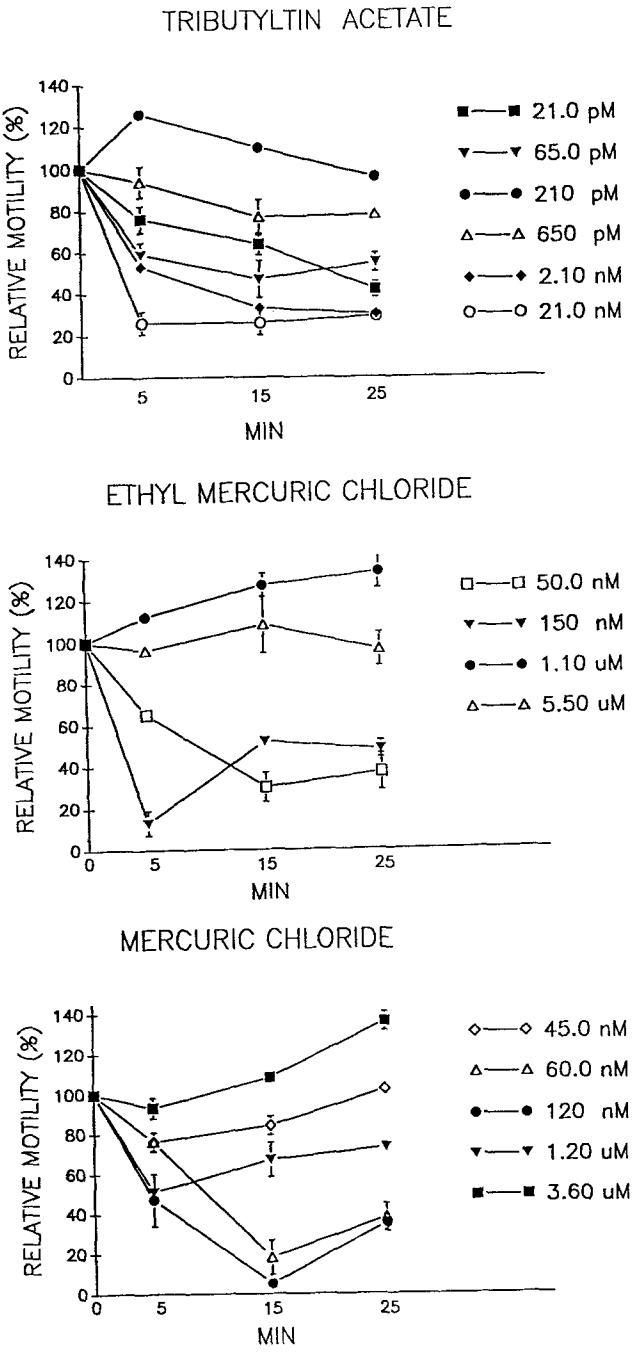
that of the controls over the 25 min incubation period. The inorganic mercuric salt, $HgCl_2$, has a strongly depressant effect, almost completely abolishing motility during the first 15 min of exposure at 120 n*M*. Incubation in 60 n*M* $HgCl_2$ evokes a closely parallel response, while at 45 n*M*, the inhibitory effect is virtually lost. Seemingly paradoxically, at the highest concentration assayed, 3.6 μM, there appears to occur a slight increase in the rate of motile progression. The marked depression of motility at the higher concentrations suggests that propagation of the flagellar wave may depend on the integrity of the sulfhydryl regulatory sites on the sperm cell's sliding filaments or on its respiratory metabolism or both (Young and Nelson 1974).

Pesticides entering the coastal environment adversely affect marine invertebrates and may also affect other marine organisms and their predators along the food chain. Isolated sperm cells, directly exposed to the noxious substances, serve as excellent in vitro indicators of the toxicants' potential in this increasingly fragile ecosystem. However, due to the fact that the pesticides tested are of limited solubility in aqueous media, they tend to aggregate and precipitate in the form of sedimentary particulates to the seafloor. The sedimented particles, of various dimensions, ingested by the sea urchins, pass through their digestive tracts, the amount of the toxicant distributed into the tissues is, at present, an indeterminate quantity; a substantial portion of the material is returned to the seabed.

Acknowledgment. Supported by the Sage Foundation, Detroit, Michigan.



Figures 2, 3, 4. Effects of organochlorine insecticides on progressive motility of sea urchin sperm cells. Fig. 2, dieldrin; Fig. 3, lindane; Fig. 4, mirex.



Figures 5, 6, 7. Effects of metal compounds on sea urchin sperm motility. Fig. 5, tributyltin acetate; Fig. 6, ethylmercuric chloride; Fig, 7, mercuric chloride.

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Received March 15, 1990; accepted April 27, 1990.