Disrupted Patterns of Behavior in Natural Populations as an Index of Ecotoxicity

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This paper examines behavioral changes in natural populations of wildlife associated with pollution. Although some changes such as lack of nest attentiveness and decreased nest defense have been noted, the results have not been consistent and have been difficult to relate to specific pollutants. Experimental studies involving lead, mercury, and organochlorine and organophosphate insecticides are described. Although changes in behavior have been observed, they are generally more difficult to quantify and are less reproducible than biochemical changes. To date, there is no clear evidence in wildlife that behavioral changes caused by pollutants are a serious threat to populations. — Environ Health Perspect 104(Suppl 2):331–335 (1996)

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Introduction

The instructions for this meeting stated that "each of the papers should feature four points. *a*) How did we get to the current status of the topic? *b*) How can we relate it to risk assessment? *c*) What are the most useful current methods? *d*) What methodological advances should we seek to make a firmer connection with policy?" The neurobehavioral toxicants considered as key agents at the meeting were lead, methyl-mercury, organochlorine pesticides, and related compounds and solvents. In this paper coverage is given of the first three agents, and in addition some consideration is given to the organophosphate pesticides.

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Abbreviations used: DDE, dichlorodiphenyldichloroethylene; AChE, acetylcholinesterase; PCBs, polychlorinated biphenyls; ALAD, aminolevulinic acid dehydratase.

Historical Aspects

Warner et al. (*1*) first suggested the use of behavioral studies to assess the impact of environmentally important agents. Studies of pharmacological agents had been carried out earlier.

The rationale for behavioral studies consists of three main points. First, the behavior of an organism represents the final integrated result of a diversity of biochemical and physiological processes. Thus, a single behavioral parameter is generally more comprehensive than a physiological or biochemical parameter. Second, behavioral patterns are known to be highly sensitive to changes in the steady state of an organism. This sensitivity is one of the key values for its use in exploring sublethal toxication. Last, behavioral measurements can usually be made without direct physical harm to the organism. With aquatic animals especially, implantation of detectors introduces problems of considerable complexity. Behavioral measurements can avoid this difficulty.

A great deal of work has been done on fish with the objective of using behavioral studies as part of the regulatory process (*2*). Atchison et al. (*2*) concluded,

> Behavioral toxicity tests can, if properly designed, be used in conjunction with the current standard tests to add ecological realism to toxicant assessments, and regulations can be made as an outgrowth of the data collected. We do not see behavioral tests taking the place of acute lethality tests, chronic full, or partial, life cycle tests, or early life stage tests.

The progress that has been made toward the use of behavioral tests in regulation is discussed below.

One of the difficulties with putting ecological realism into tests is that the behavioral tests themselves often lack ecological realism and are rarely carried out with the target species. The two most sensitive indicators of sublethal exposure in fish are the cough rate and the avoidance reaction. While an increase in the cough rate can be considered an adverse effect, it is hard to quantify cough rate in relation to adverse outcome; an increase in coughing by humans may be caused by increased air pollutants, but it would be hard to make regulations on the basis of it. The avoidance reaction could be a valuable defense mechanism for fish. Indeed, Saunders and Sprague (*3*) reported that Atlantic salmon (*Salmo salar*) avoided areas contaminated with copper and zinc. However, field studies are few, and Geckler et al. (*4*) found that standard laboratory toxicity tests failed to predict the avoidance response observed in fish at copper concentrations that caused no observable effect in the laboratory.

Field Studies of Behavioral Changes Caused by Pollutants

Experimental studies have raised the possibility that behavioral changes are caused by pollutants, but the demonstration of actual effects under natural conditions is much more difficult. Field studies may be divided into two broad categories—those investigating the effects of contamination that have already occurred and those investigating effects caused by adding a pollutant to the environment as part of the study.

Studies of Preexisting Contamination

The major difficulty with field studies of this type is to prove that the effect, once demonstrated, is actually caused by the chemical(s). This difficulty is illustrated by the examination of a few of the available field studies.

Lack of Nest Attentiveness

There were two series of studies on herring gulls (*Larus argentatus*) in the Great Lakes (*5,6*). These were detailed studies on incubation behavior and an evaluation of the intrinsic and extrinsic factors by egg-exchange experiments. An initial observation on the Great Lakes was that gulls in the highly contaminated colonies in Lake Ontario tended to readily leave their colonies at the approach of observers. This observation led to detailed studies on the nest attentiveness of herring gulls on Scotch Bonnet Island at a time when reproductive success was low (*5*). Dummy eggs that contained devices to measure the core and surface temperature and whether the egg was covered were placed in the nest; these dummy eggs could transmit this information to a recorder. This battery-powered recorder unit was capable of operating for 30 days without recharging. Fox et al. (*5*) found that nest air temperature was lower on Scotch Bonnet compared to a lightly contaminated site (Kent Island, New Brunswick). More important than the modest decrease in mean temperature was the fact that there was a significant number of observations in which the temperature was lower than the physiological zero on the contaminated site. There was also a decrease in the percentage of time that the eggs were incubated on the contaminated site compared to the control site. It is difficult to be certain of the cause; it is possible that ease of obtaining food, rather than chemical contamination, caused the difference between nest attentiveness in the Lake Ontario and marine colonies.

In Lake Michigan, where the colonies were also highly contaminated, Ludwig and Tomoff (*6*) noted that the gulls were more aggressive than usual, although no details are given. It is possible that colony size and the history of the disturbance of the colony by man are important in determining whether birds readily leave the colony (P Mineau, personal communication).

Egg-Exchange Experiments

Exchanges of eggs are made between two colonies, one of low contamination (clean) and one of high contamination (dirty). Eggs are removed from the clean colonies and placed under adults in the dirty colony, and vice versa. The outline of the experiment is in Table 1.

Table 1. Outline of egg-exchange experiment.

Adult	Egg	Observation
Clean	Clean	Normal reproduction
Clean	Dirty	Intrinsic factors only
Dirty	Clean	Extrinsic factors only
Dirty	Dirty	Both intrinsic and extrinsic factors

In concept, egg-exchange experiments are simple enough, but in practice all sorts of complications occur. Obviously, it is necessary to demonstrate that there are no serious effects from transportation. Then, the timing of breeding can vary markedly from colony to colony. In the Great Lakes herring gull experiment (*7*), marked intrinsic and extrinsic factors were noted in 1975, marked intrinsic and some extrinsic factors in 1976, and no effects at all were noted in 1978. The reason for this apparent inconsistency is that the reproductive success of herring gulls on Lake Ontario was improving rapidly over this period.

Egg-exchange experiments have also been carried out with the osprey (*Pandion haliaetus*) (*8*). These workers found that the hatching success of dirty eggs from Connecticut was not improved by placing them under clean adults in Maryland and that clean eggs placed under dirty adults hatched at their normal rate. Wiemeyer et al. (*8*) concluded that the effects were intrinsic to the egg.

Changes in Nest Defense

Fyfe et al. (*9*) found that a decrease of nest-defense behavior of both prairie falcons (*Falco mexicanus*) and merlins (*Falco columbarius*) was significantly correlated with both the degree of eggshell thinning and egg residue levels in the eggs. Nest defense of the adult was classified as aggressive if one or both of the adults made direct attacks on the intruding investigator, moderate if one or both adults were vocally aggressive but did not attack, weak if both adults left the immediate area of the nest, and absent if both adults were absent at the time of the visit. These workers did not attempt to evaluate the importance of change in nest-defense behavior compared to other pollutant effects in the decrease of nesting success.

In another study of the effects of organochlorines on nest-defense behavior of merlins (*10*), only minor behavioral changes were noted; these authors concluded that changes in behavior are of minor importance in the reproductive failure of falcons associated with dichlorodiphenyldichloroethylene (DDE) contamination. The methodology used in this study differed from that of Fyfe and co-workers (*9*); it depended on the incidence of stooping and vocalization during a mid-incubation visit and the response of the adults to a tethered merlin or other hawks. It appears that the criteria of Fox and Donald (*10*) are largely a subdivision of the Fyfe et al. (*9*) aggressive category, which may account for the differences between the two studies.

Behavioral Component of Egg Breakage

Eggshell thinning induced by DDE has been shown to be an important factor in the decline of several species of birds of prey, especially the peregrine (*Falco peregrinus*). It has been demonstrated for a wide spectrum of populations of peregrines that eggshell thinning of more than 17 to 18% is associated with population declines (*11*). Ratcliffe (*12*) stated that "the implications are that, in the British peregrine, which has suffered a 19% decrease in eggshell thickness, some of the breakage may be behavioral and [may] occur without prior mechanical damage as a stimulus." This opinion was based on observations of the grey heron (*Ardea cinerea*) by Milstein et al. (*13*). Although this paper is widely cited, the observation of deliberate breaking of eggs was confined to one nest. In this nest, 21 eggs disappeared, and only 2 of these were actually observed to be destroyed by the male.

Studies on the peregrine have failed to reveal firm evidence of behavioral abnormalities. Time-lapse photographic records were made of seven eyries in Alaska in 1970 (*14*). Enderson and co-workers (*14*) used battery-powered, time-lapse motion picture cameras to take pictures about every 3 min; the film cartridges needed to be replaced every 6 to 7 days. Even so, it was a full-time job to replace the film cartridges as the eyries were widely separated and the terrain was difficult. In two of the nests, the eggs broke but no evidence of abnormal behavior was observed. The other five nests were successful. In all, some 70,000 pictures covering 4,200 hr were obtained. One of the drawbacks of this type of experiment is the time taken to analyze the data.

Observations from a blind, totaling over 300 hr, were made on 12 clutches of peregrines in British Columbia between 1968 and 1972 (*15*). Of these 12 clutches, 4 clutches lost single eggs, apparently by breakage, but no abnormal behavior was observed. Although 300 hr is a lot of time to spend sitting in a blind, it is only 25 hr per clutch out of 400 hr of daylight during the incubation period.

Conclusion

While much interesting information has been accumulated by these studies, the evidence is weak that behavioral changes are important factors in adverse effects on wildlife caused by pollutants. Even where behavioral changes have been identified, it has rarely been possible to relate these to an adverse effect; it is even more difficult to make the linkage to a specific chemical or chemicals.

Experimental Field Studies

When a wild population is experimentally exposed to known chemicals, it is possible to establish a cause-and-effect relationship rather than mere correlations. Nevertheless, there are serious difficulties in carrying out such experiments. The first difficulty is finding an acceptable way of exposing the target organism to the pollutant. Animals are normally contaminated through diet, but this is difficult to use. Tethering of contaminated prey was used by Enderson and Berger (*16*) in studies on falcons, but it is doubtful if this technique would be acceptable to present day animal-care committees. Injection has the advantage of containing the pollutant, although some may be excreted, but is an entirely unnatural route. Dosing *per os* to simulate dietary intake has been used for experiments on the effects of oil on seabirds (*17*), and implants of tubes containing polychlorinated biphenyls (PCBs) (*18*) have been employed to allow slow release. However, none of these studies had a behavioral component.

The second major difficulty is that of making detailed observations. Handling the animals and submitting them to carefully calibrated tests is likely to cause so much disturbance that there will be serious effects from this cause alone. Remote observation, either from a hide or by camera, is also difficult. Subtle behavioral changes are difficult to observe, let alone quantify, and a large amount of labor is involved with either sitting in a hide or examining large rolls of film.

Last, there is the problem of relating any changes observed to adverse effects. Even if significant changes are observed and adverse effects are demonstrated, it still remains to be proven that the behavioral changes are caused directly by the pollutant rather than by other changes, such as physiological changes.

No experimental studies appear to have been made in the field involving lead, methylmercury, or organochlorine pesticides in birds. The best experimental studies are those involving the organophosphorus and carbamate pesticides. In this case, operational usage allows experimental studies to be made.

Experimental Studies on Wildlife Species Using Key Agents

Lead

A series of studies have been made on postnatal development of the herring gull and the common tern (*Sterna hirundo*) by Burger and Gochfeld (*19–21*). In the herring gull experiments (*19*), 1-day-old chicks were brought into the laboratory and given a single injection of lead nitrate. The behavioral tests examined balance, begging, locomotion, righting, and visual cliff. Although on most days behavior (begging, balance, and righting responses) did not differ significantly, over the entire period control birds performed better on more days than experimental birds. Individual recognition was delayed in a dose-dependent manner by injected lead. The residue levels of lead associated with this treatment were given in a subsequent paper (*20*).

A wider range of doses was used in the experiments on terns (*21*). Single injections of 0.2 to 1 mg/g were given to 3-day-old chicks. The highest dose caused 50% mortality. Using two injections 4 days apart, 100% mortality was caused by injections of 0.3 mg/g, whereas with injections of 0.2 mg/g, the mortality was only 20%. The injected route ensures that the dose is delivered to the bird but bears no relation to the natural route of exposure. These workers found that behavioral tests were affected in a dose-dependent manner, and some effects were seen at the lowest dosage used, which was a fifth of the LD_{50}.

The difficulty with these types of experiments is that it is impossible to relate the behavioral changes to any adverse effects on the population as a whole. It can only be assumed that a behavioral change is likely to be detrimental to the survival of the individual. Testing this hypothesis would be difficult. As stated earlier, making detailed observations, either from hidden sites or by remote-control cameras, is time-consuming compared to laboratory experiments. The major difficulties are avoiding observer-disturbance effects and having a large enough sample size to overcome the high natural mortality.

Methylmercury

The most detailed study has been that of Heinz (*22*), who exposed mallard ducks (*Anas platyrhynchos*) for three generations to low levels (0.5 ppm) of dietary methylmercury. The behavioral tests studied were approach responses of ducklings to maternal calls, avoidance responses to a fright stimulus, and open-field activity. Additionally, reproductive parameters were measured. Ducklings from parents fed methylmercury were less responsive than controls to maternal calls, showed a greater response to the fright stimulus, and displayed no significant differences in locomotor activity in the open-field test. No differences were found between the responses of the first, second, and third generation. This correlates with the fact that residue levels of mercury did not increase from generation to generation. Some effects were also seen on reproduction; mallards exposed to methylmercury laid a greater percentage of their eggs outside their nests, laid fewer eggs, and produced fewer ducklings. Overall, there was a statistically significant reduction of ducklings raised in the group exposed to mercury compared to controls.

Since the experiment was carried out in the laboratory, the behavioral changes observed would not have affected reproductive outcome. Thus, the reductions seen were the direct effects of the methylmercury; the additional effects that might have been caused in the wild by the behavioral effects cannot be ascertained. Heinz (*22*) concludes,

> The tissues and eggs of ducks and other species of birds collected in the wild have sometimes contained levels of mercury equal to or far exceeding the level I found to be associated with reproductive and behavioral aberrations. Therefore, it is possible that reproduction and behavior of wild birds has been affected by methylmercury contamination.

Cholinesterase Inhibitors

Although not one of the key agents considered at the meeting, the cholinesterase inhibitors (organophosphorus and carbamate pesticides) are included here because adverse effects are possible, and some field experiments have followed the operational use of these chemicals.

The inhibition of the enzyme acetylcholinesterase (AChE) has been clearly related to the mode of toxicity of organophosphates and carbamates, and the degree of inhibition has been related to mortality. In avian species, at least, mortality has been related to 50% chronic or 80% acute inhibition of AChE (*23*). Inhibition of AChE is used as the diagnostic tool to ascertain toxicity of these pesticides; thus, it seems reasonable to relate behavioral changes to the degree of inhibition in order to determine the relative sensitivity of the two approaches. In a previous review (*24*) it was concluded that "for organophosphates the evidence is clear-cut that cholinesterase inhibition is a more sensitive and more readily determined parameter of exposure than are behavioral changes." In a recent paper, Hart (*25*) discussed experiments on starlings (*Sturnus vulgaris*) exposed to chlorfenvinphos, and also reviewed the field in some detail. He examined the thresholds for behavioral effects and concluded that quantitative changes of behavior have been observed at 50% reduction of AChE activity, but not at 25% reduction from normal levels. Hart considered it unlikely that the relationships established between AChE activity and behavior in the laboratory could be used as reliable predictors of effects on wild birds. His main reservation was that behavior in the wild is adaptive and context dependent. Hart concluded that "studies of captive and free-living birds provide general support for the proposition that a change in behavior can be expected when brain AChE activity falls below about 50% normal."

Summation

Three points were put forward by Warner et al. (*1*) as the rationale for behavioral studies: *a*) a single behavioral parameter is generally more comprehensive than a physiological or biochemical parameter because it represents the final integrated result of a diversity of biochemical and physiological processes; *b*) the sensitivity of behavioral patterns is one of the key values for its use in exploring sublethal toxication; and *c*) behavioral measurements can usually be made without direct physical harm to the organism.

While it is true that a single behavioral parameter is more comprehensive than physiological or biochemical parameters, it is the complexity of the behavioral responses that seems to have caused the major difficulty in quantifying them so that they can be used to assess pollution effects. The second point has not withstood the test of time: in general, behavioral effects have not been shown to be more sensitive than measurements of biochemical or physiological processes and, further, they tend to be more time consuming and less reproducible. The last point, that behavioral measurements can usually be made without directly harming the organism, certainly seems to be forward looking. There has been increasing emphasis on nondestructive testing in recent years. A recent symposium has been devoted to nondestructive biomarkers in vertebrates (*26*); however, behavioral biomarkers are given only one brief mention in the concluding chapter.

It seems likely, especially in higher organisms, that other pathways and strategies can be used to overcome the damage done by chemical insult. This seems to be particularly likely when the behavior studied is vital to the organism. For example, experiments on the prey-capturing ability of American kestrels (*Falco sparverius*) dosed with the organophosphate acephate failed to show any effect, either alone or in combination with DDE, despite the fact that 40% inhibition of serum cholinesterase was observed (*27*).

There has been the feeling that subtle behavioral changes in wild populations could cause serious effects at low levels of pollutants, which did not have effects on mortality or reproduction. This concern has been the driving force for many studies. So far, these fears appear to have been groundless. To date, behavioral tests on wild species have not been used in regulations to provide data to be used in risk assessment. Tests are most advanced for aquatic organisms. In examining the effects of pollutants on wild populations, methods will certainly have to be tailored to the specific problem being addressed. The balance between operant tests, which are easy to control but difficult to interpret, and more realistic tests that are difficult to control is not an easy one. Perhaps the best way forward here is to determine, using operant tests, if effects are seen at environmentally realistic dosages before proceeding on to more complex tests. In view of the likelihood that higher organisms can compensate for chemical insults when a vital behavioral function is involved, a move toward using less complex organisms might be advantageous. Two possibilities would be the signaling behavior of bees and the web-building of spiders. The latter is discussed in more detail by Cohn and MacPhail (*28*). A detailed comparative study, covering a wide range of phyla, on the behavioral effects of a single chemical (probably an acetylcholinesterase inhibitor) would be valuable. Even in laboratory studies, we have a long way to go before behavioral studies can be used for regulatory purposes. It is important that all behavioral studies of the effects of pollutants be related not only to dose but also to residue levels so that the effects seen can be related to levels found in nature. Further, it would be valuable if behavioral changes were linked to biochemical biomarkers. There is a movement toward using biochemical biomarkers in regulatory processes; already, dioxin equivalents have replaced the determination of dioxins themselves. The following biomarkers are proposed as being well enough established to be used for comparison with behavioral changes: organophosphates and carbamates, inhibition of AChE; lead; inhibition of aminolevulinic acid dehydratase (ALAD); organochlorines, induction of mixed function oxidases (including the calculation of dioxin equivalents); and, for petroleum hydrocarbons, the induction of mixed function oxidases as well.

Malins and Ostrander (*29*) state that "behavioral toxicology continues to receive modest attention among aquatic toxicologists." Nevertheless, even a condensed review of behavioral toxicology requires a book on the scope of the current workshop. Here I will conclude with a brief review of recent statements covering a number of different phyla. Doving's (*30*) review of animal behavior as a method to indicate environmental toxicity concludes that "behavioral toxicology will gain momentum in the years to come." He considers that the behavior of fish larva (despite the broad title the review is devoted exclusively to fish) should be given top priority. Another review on fish (*31*) concludes, "There is a strong need for interdisciplinary studies focusing on a few substances and a few selected fish species." Haynes (*32*) focused on the sublethal effects of neurotoxic insecticides on insect behavior. He stated in his concluding remarks that "neurotoxic insecticides may adversely affect all elements of the behavioral repertoires of insects at doses much lower than the lethal dose," but he did not give any specific examples. Indeed, he stated that "there have been few detailed studies concerning the potential behavioral effects of sublethal doses of insecticides." To finish on a more optimistic note, Janssen et al. (*33*), discussing the behavior of freshwater rotifers, concluded that "it can be stated that toxicant induced changes in the swimming and feeding behavior are reflected in the demographic parameters at approximately the same toxicant levels" and that "the time needed to perform routine chronic toxicity tests could be dramatically reduced while still retaining its ecological relevance."

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