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Forensic Ecotoxicology: Establishing Causality between Contaminants and Biological Effects in Field Studies

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ABSTRACT

Twelve papers in this series were derived from two conference sessions focusing on causality in field studies. Eight of these papers involve case studies examining biological effects of chemical contaminants in field situations. Using a weight-of-evidence approach, these case studies were evaluated against seven proposed criteria for establishing causality. The seven criteria were: strength of association; consistency of association; specificity of association; time order; biological gradient; experimental evidence; and biological plausibility. One of these seven criteria, 'specificity of association' was found to be of little utility for establishing causality in these field studies. The case studies are presented in approximate order of increasing levels of biological organization (*i.e.*, going from endpoints at the suborganismal level to endpoints at the population or community level). In case studies examining higher levels of biological organization, it appears that the 'biological gradient' criterion was also not useful in establishing causality. These results, together with suggestions from other papers in the series, are used to recommend a set of modified criteria for establishing causality in field studies of the biological effects of chemical contaminants.

Key Words: causality, ecoepidemiology, weight of evidence, field study, contaminants.

INTRODUCTION

A major aim of environmental toxicologists is to establish causal linkages between exposure to a toxicant, or toxicants, and resultant biological effects. In the laboratory, where investigators study the relationships between exposure to

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limited numbers of toxicants and alterations in limited numbers of endpoints, such linkages can be examined exhaustively. Laboratory replication and dose-response studies allow definitive statements concerning the nature of causal linkages. However, when these linkages are sought in natural ecosystems, many other factors may interfere with our ability to make definitive statements concerning causality. These other factors include the multitude of toxicants often present in natural systems, non-contaminant stressors (including both anthropogenic and natural stressors), and the inherently high biological variability of natural systems. The term 'forensic ecotoxicology' can be used to describe efforts to determine causality resulting from exposure to toxicants outside of controlled laboratory studies. To date, there are no widely accepted approaches for establishing causality in natural ecosystems (Adams 2003). In this issue of the *Journal of Human and Ecological Risk Assessment* (HERA), eight authors present case studies where attempts were made to determine causal relationships in natural ecosystems, focusing on environmental contaminants as the primary stressor(s). As a precursor to their case studies, Adams (2003) proposed seven causal criteria, which each author was requested to use in evaluating their particular case. These seven criteria were derived from Koch's postulates, as well as earlier causal criteria lists and proposals, including those put forward by Hill (1965), Fox (1991), Suter (1993), and the U.S. Environmental Protection Agency (USEPA 2000). The seven criteria, and brief descriptions of each, are given below. Criteria marked with an asterisk are described somewhat differently than originally proposed by Adams (2003), due to subsequent discussions.

* 1. Strength of association—there is a strong relationship between the stressor and the effect. For example, either a large proportion of individuals in a stressed area is affected, or perhaps a small proportion of individuals shows a large effect. Alternatively, there may be a strong relationship seen between an effect in individual organisms and the presence or absence of a supposedly causal toxicant in their bodies.

2. Consistency of association—the relationship between the stressor and the effect has been seen in other studies, especially in studies by other investigators.

3. Specificity of association—the effect is diagnostic of exposure to the stressor, meaning that the effect is only observed after exposure to that stressor. Additionally, the stressor produces only that effect.

* 4. Time order/temporality—the effect occurs only after exposure to the stressor. Alternatively, does removal of the stressor result in a subsequent reduction in the effect?

5. Biological gradient—there is a dose-response relationship between the stressor and the effect. As the magnitude of the stress increases or decreases, so does the magnitude of the effect.

6. Experimental evidence—controlled exposure to the stressors (*e.g.*, in laboratory or mesocosm studies) provide results that support the proposed causal relationship.

7. Biological plausibility—The proposed causal relationship has a credible biological (or in this case toxicological) basis. Mechanistic linkages can be proposed linking stressor exposure to the biological effect.

Eight of the authors in this series of papers evaluated their case studies for causality using the above criteria. Table 1 presents summary information for each of the eight case studies. The objectives of this final paper in the series are to evaluate the eight case studies against the causality criteria, determine the relevance of each criterion in establishing causality based on consideration of all eight case studies taken together, and provide a prospectus for future developments in forensic ecotoxicology.

EVALUATING THE CASE STUDIES AGAINST THE CAUSALITY CRITERIA

Each of the case studies were evaluated relative to how well their results matched each of the causal criteria (Table 2). The case studies were ranked in approximate order of increasing levels of biological organization (*i.e.* going from endpoints at the suborganismal level to endpoints at the population or community level). Two of the case studies [Moraes *et al.* (2003) and Triebskorn *et al.* (2003)] considered biological effects at several levels of organization, but were the only studies that also examined

Table 1. Summary information for the case studies presented in this series, and evaluated in Table 2.

Case study	Endpoint(s)	Organisms	Contaminant(s) linked to causality	Comments
Theodorakis (2003)	Genetic alterations	Fish	Radionuclides	Freshwater ponds. Multi-generational exposure.
Schlenk (2003)	Biochemical alterations	Fish	PCBs	River system. Example of applying causality criteria to a limited dataset.
Myers *et al.* (2003)	Liver histopathology	Fish	PAHs	Open marine system. Remedial actions used to test temporality. Decades of field data.
Brown *et al.* (2003)	Reproduction	Clams	Silver	Open estuarine system. Reduced exposure used to test temporality. A decade of field data.
Halbrook and Arenal (2003)	Reproduction	Birds	PCBs	Terrestrial Superfund site. Remedial actions used to test temporality.
Brown and Fairchild (2003)	Populations (catch rates)	Fish	Insecticide formulation	Riverine system. Reduced catch of returning adults several years after exposure of juveniles.
Moraes *et al.* (2003)	Biochemical to community	Fish	Metals	Tropical rainforest system.
Triebskorn *et al.* (2003)	Biochemical to community	Fish and benthos	General mixture of contaminants	Small stream systems. Multi-institutional collaboration.

Table 2. Evaluation of case studies against causality criteria proposed by Adams (2003). (+++) Convincing evidence presented that the criterion was met. (++) Strong evidence presented, yet some questions raised. (+) More likely than not, or only little evidence presented. (+/-) Evidence presented both for accepting and rejecting criterion, or no evidence available regarding that criterion. (–) Evidence presented argues against accepting the criterion. (NA) Criterion not addressed.

	Theodorakis (2003)	Schlenk (2003)	Myers *et al.* (2003)	Brown *et al.* (2003)	Halbrook and Arenal (2003)	Brown and Fairchild (2003)	Moraes *et al.* (2003)	Triebskorn *et al.* (2003)
1. Strength of association **(15)**[§]	+	++	+++	++	+++	+++	+	+/-
2. Consistency of association **(17)**	+/-	++	+++	++	++	+++	+++	++
3. Specificity of association **(2)**	+	+	+	+/-	+/-	+/-	–	+/-
4. Time order/ Temporality **(11)**	++	+/-	++	++	++	++	NA	+
5. Biological gradient **(15)**	+++	++	+++	+++	++	++	+/-	+/-
6. Experimental evidence **(15)**	+++	++	++	++	+++	+	NA	++
7. Biological plausibility **(18)**	++	++	+++	++	+++	+	++	+++
Estimated score[#]	**12**	**11**	**17**	**13**	**15**	**12**	**(5)***	**8**

§ Number following each criterion is the estimated score for that criterion.

The estimated score for each case study. Estimated scores were derived by summing the rankings for the individual case studies and causality criteria as follows: (+++) = 3; (++) = 2; (+) = 1; (+/-) = 0; (–) = -1.

* Not all causality criteria addressed.

possible effects at the community level. For their evaluations, only effects at the community level were considered. All studies were evaluated in reverse alphabetical order, using the surnames of the primary authors, to reduce bias that might derive from a structured sequence of evaluation. For example, Brown and Fairchild (2003) was evaluated before Brown *et al.* (2003). To score the case studies against the criteria, a +/- system was used, as follows:

+++ Convincing evidence presented that the criterion was met

++ Strong evidence presented, yet some questions raised

\+ More likely than not, or only little evidence presented

+/– Evidence presented both for accepting and rejecting criterion, or no evidence available regarding that criterion

– Evidence presented argues against accepting the criterion

NA Criterion not addressed

While the evaluation was not designed to be quantitative, approximate scores can still be estimated by giving a score of 1 for each (+), 0 for each (+/–), –1 for each (–), and summing each case study across all criteria, and each criterion across all case studies, as shown in Table 2. Overall, the most striking observation from Table 2 is that criterion 3, specificity of association, was the least applicable to the case studies, with an estimated total score of 2. Time order had the second lowest rank, with a total score of 11. The totals for the other five criteria ranged between 15 and 18. Another notable result is that the case studies at the community level tended to have lower causality rankings than those case studies at lower levels of organization, and the community studies also were comparatively weaker than other case studies in meeting the 'biological gradient' criterion. The preceding results are instructive for investigators seeking to establish causality in field studies. They suggest that the criterion for 'specificity of association' appears to be of little use for studies in natural systems. The low scoring for this criterion is likely due to the presence of multiple stressors, including both contaminant as well as non-contaminant stressors, in virtually all ecosystems which have been degraded by human activities. It is difficult to link a specific stressor with a specific effect not only because of the presence of multiple stressors, but also because stressors can interact with each other in synergistic, antagonistic, or additive fashion. Based on the examination of causality criteria in these studies, it appears that using 'specificity of association' for establishing causality in field studies is not warranted. Time order, or temporality, likely receives a lesser score in these studies because of the difficulty in controlling temporality in field situations, with the exception of remedial efforts. Quite often field studies involve conducting a retrospective risk assessment, where a study is initiated because a biological effect has been noted, and the field study is designed to find the cause. Of the several types of ecological risk assessments, the retrospective risk assessment is often deemed the most difficult (Suter 1983).

The relatively low causality scoring for the community level endpoints is not surprising, as many authors have noted the difficulty in establishing causal links to altered community metrics. Higher-level responses, such as population and community endpoints, are integrative in nature, reflecting the influence of multiple environmental factors over long time scales. Recently, Johnson and Collier (2002) made the argument that regulatory approaches that require evidence of biological effects at population or community levels may be misguided, specifically because of the difficulty in establishing causal linkages to higher levels of biological organization. These investigators further suggest that "in order to be proactive in the conservation of our….resources, it may be appropriate to use regulatory approaches that protect the health of individual organisms from the effects of anthropogenic stress". While there are some well-documented case studies where community level alterations have been causally linked to simple sets of stressors in field studies (Adams *et al.* 2002; Suter *et al.* 1996), such clear relationships should not be expected in most studies dealing with natural systems and multiple stressors. It is interesting to note that the studies evaluated in Table 2, which looked at impaired indices of individual health (reproduction and histopathology in invertebrates, fish, and birds) tended to receive the highest causality rankings, even more so than for the suborganismal studies. This may be because each of these studies included a temporal aspect, resulting from reduced inputs, or remedial activities. While it is tempting to analyze this data set further, and draw more inferences, the subjective nature of the scoring, as well as the case study selection process, argues against further quantitative analysis.

ADDITIONAL CONSIDERATIONS IN FORENSIC ECOTOXICOLOGY

Three additional papers included in this series address separate aspects of causality that investigators should be aware of when conducting field investigations of causality. The paper by Hewitt *et al.* (2003) presents a plan for establishing degrees of causality in field studies, because for many situations, it may not be necessary, nor affordable, to attempt to determine precise causality. They propose that the level of a causality investigation needs to be established with inputs from stakeholders as well as considerations of the costs and complexities of field studies that can establish causality. Their approach starts with first establishing that there is indeed an effect occurring in the environment, and moves on through increasing levels of effort to link effects with specific effluents, processes, and eventually to specific chemical stressors. Norton *et al.* (2003) discuss several types of cognitive errors that can occur in interpreting studies of site-specific causality, and suggest general rules for avoiding such errors. For example, while the case studies in this issue are focused on contaminants as primary agents of effects, investigators should realize that other non-contaminant or even nonanthropogenic stressors could, in some situations, elicit the same biological responses. This tendency increases with increasing levels of biological organization, as mechanistic understanding decreases. Thus, Norton *et al.* (2003) strongly recommend that investigators explicitly consider alternative causal agents in the evaluation of their data, and in fact in designing their studies. Finally, Rose *et al.* (2003) suggest an additional approach to establishing causal relationships between contaminant exposures and population level effects in fish.

They elegantly describe and demonstrate the use of nested models, and how these models can be used to extrapolate laboratory studies to effects at the population level. Through this process, insight into causal mechanisms and linkages is enhanced. This approach could provide investigators with plausible linkages and relationships to focus on, and confirm, in field studies.

PROSPECTUS FOR FORENSIC ECOTOXICOLOGY

There are six causal criteria that should be useful in most field studies of the biological effects of chemical contaminants. These are as follows: strength of association, consistency of association, time order, biological gradient, experimental evidence, and biological plausibility. These are derived from the list of seven put forward by Adams (2003), minus one criterion (specificity of association), which was found, based on the eight case studies, to be of little utility in establishing causality in studies of natural systems. The criteria are defined earlier in this paper, and using them constitutes a weight of evidence approach to determining causality. However, there are areas where the definitions may overlap, such as between strength of association and biological gradient. To address overlaps, and to provide specific examples of datasets that meet the criteria, it would be useful for a group of investigators to better define the criteria, using examples taken from published literature.

Remedial actions undertaken to remove chemical contaminants from natural systems provide an excellent opportunity to add a temporal component to field studies of causality, and this temporal component can be readily achieved by monitoring the effects of remedial actions. However, due to the cost of monitoring, and the desire to use funds for further cleanup actions rather than monitoring of completed actions, long-term monitoring programs to assess the biological effectiveness of remedial actions are not commonly implemented. Nonetheless, such monitoring efforts may provide our best opportunity to increase our understanding of putative causal relationships.

As forensic ecotoxicological investigations move to higher levels of biological organization, it will be more difficult to establish causality especially when multiple stressors are involved. There are several reasons for this, but primary factors are the complexity of ecological linkages in natural systems, associated difficulty in experimental replication, the integrative nature of higher-level responses and compensatory mechanisms. Modeling approaches may help establish causality at higher levels of biological organization. However, inability to demonstrate causality at the population or community level should not be taken as proof that no causal relationship exists. In order to protect our natural systems from the effects of multiple stressors, arguably different levels of causal certainty, inversely correlated with the level of biological organization of the endpoints of concern, will be needed to support regulatory actions.

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