The Usefulness of Cholinesterase Measurement: A Response

Author: Hill, Ellwood F.

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RESPONSE TO LETTER TO THE EDITOR . . .

The Usefulness of Cholinesterase Measurement: A Response

The letter of Fairbrother and Bennett (1988) in regard to my paper"Brain cholinesterase activity of apparently normal wild birds" (Hill, 1988) and the usefulness of cholinesterase (ChE) measurements indicates a need to clarify certain conceptual and technical details pertinent to evaluation of wildlife exposure to the antiChE carbamate and organophosphorus pesticides. First, this paper was a practical sequel to earlier publications from the Patuxent Wildlife Research Center (Center) on experimental diagnosis of antiChE poisoning (Ludke et al., 1975) and use of such diagnostic criteria in the field (Hill and Fleming, 1982). It was based on study of 83 sets of specimens of 48 avian species provided for reference in the investigation of diverse episodes of wildlife mortality. These "controls" were collected opportunistically by investigators from across the USA and transported to the Center under variable conditions of refrigeration and freezing. Although prompt freezing was preferred for toxicological evaluation, most specimens were initially chilled and examined for infectious disease before being frozen for transmittal to our Center. Occasionally frozen specimens thawed during transit. All specimens were stored at −25 C for at least 3 days at the Center prior to performance of ChE assay under a common protocol. In spite of temporal inconsistencies in storage, other unaccounted irregularities, and even possible antiChE exposure of some individuals, close agreement was consistently demonstrated among multiple sets of control specimens of a given species provided to the Center over several years (Hill, 1988; Table 2). None of the within-species comparisons were statistically separable (one-way ANOVA, $\alpha = 0.05$) and the critical diagnostic level of ChE activity (50% of control mean; Ludke et al., 1975) never varied more than two activity units within any of the six species compared. Thus, evaluation of 83 sets of unrelated control submissions provided information on species differences in whole brain ChE activity and a spectrum of ChE activity estimates for apparently healthy wild birds for use with appropriate caution solely at the considered discretion of the investigator. Two main conclusions were therefore offered: (1) "I encourage the use of presented (ChE) values as *emergency* substitutes in diagnosis of *lethal* anticholinesterase poisoning when concurrent controls *cannot* be obtained," and (2) ". . . the (ChE) values *are* reproducible, provided the described procedures including reaction temperature are duplicated. . . ."

Prospective users of ChE values in Table 1 must first conduct tests on several common species to insure interlaboratory agreement of results. This step could be enhanced with assays of a ChE standard for quality assurance and interlaboratory interpolation as suggested in our initial report (Ludke et al., 1975) and recommended by Fairbrother and Bennett (1988). Specific comparisons should be primarily used for diagnosis of lethal antiChE exposure and only for tentative indication of sublethal exposure. This is because depression of 50% in whole brain ChE activity was experimentally correlated with death from antiChE exposure, but depression in field-killed specimens usually exceeds 70%, and thereby provides a practical buffer against erroneous diagnosis. In contrast, when the objective is to monitor sublethal antiChE exposure of free-ranging wildlife, detection of more subtle (e.g., <30%) ChE depression requires the best possible estimate of the variance of control ChE activity and is most reliably based on concurrent controls collected and processed identically with the subject specimens in accordance with preferred institutional methods. This latter approach or use of a

cumulative institutional data base is recommended for all planned research. Although significant antiChE exposure can be determined for individuals by comparison to normal values for the species (i.e., ChE activity two standard deviations below the mean; Hill and Fleming, 1982), use of parametric comparisons are preferred for comparing treatments.

Fairbrother and Bennett (1988) suggest a data base such as offered in Table 1 ". . . is likely to be used in regulatory decisions setting guidelines for acceptable amount of ChE depressions caused by proposed pesticide use." Such use may be inappropriate because very little is known about the biological implications of altered whole brain ChE activity other than it is usually depressed more than 50% in animals that die of antiChE exposure. Otherwise, significantly depressed ChE activity simply infers recent (e.g., 2 to 4 wk; Fleming and Grue, 1981) exposure to an antiChE substance. Moreover, individuals may survive with ChE depressed more than 50% from a given antiChE exposure while cohorts die, and survivors cross the same graded levels of depression during recovery as initially experienced from exposure (Ludke et al., 1975). Although individuals are undoubtedly at different levels of risk during ChE decrease and increase, the direction in which change is proceeding cannot be determined by simple whole brain ChE assay in free-ranging animals. Correlation with blood ChE activity and detection of antiChE residue may be helpful in determination of direction of change, a topic beyond the scope of the present comment. Even when the time and rate of an application of antiChE pesticide is known, it is difficult to document whether a given free-ranging individual with significant antiChE depression was actually exposed in the treated locale. Also, consequences of a given level of brain ChE depression vary according to the rate and number of exposures received. These comments are not intended to discourage the use of whole brain ChE activity in research, but are cautions against possible erroneous interpretation of what constitutes "acceptable amounts of ChE depression." At this time, it may be best to continue to use whole brain ChE activity primarily as a quantal tool in detection of exposure to or diagnosis of death from antiChE pesticide. Instead, I encourage research be done on ChE activity in discrete regions of the brain which may provide more meaningful interpretation of the potential effects associated with graded ChE depression.

The importance of consistency in specimen collection, storage, processing and biochemical methodologies cannot be overstated. Fairbrother and Bennett (1988) have commented on certain methodologies that can significantly alter rates of ChE activity and have suggested that most steps from specimen collection through final assay be standardized. Many suitable assay techniques (e.g., colorimetric, titrimetric, radiometric, manometric, electrometric, etc.) are presently in use. However, because the main correlative in evaluation of antiChE exposure is the degree of depression in relation to the normal value for the individual or species, the specific method of choice should be the prerogative of the respective laboratory.

In conclusion, the concerns raised by Fairbrother and Bennett (1988) in use of an uncontrolled data base of whole brain ChE activity are valid and have been long recognized at our Center. We do not rely on such data for controlled research, but have effectively used our institutional data base in resolution of field incidents of wildlife mortality of unknown origin. In this latter situation, the ability to improvise is essential and justifiable because statistical evaluation of multiple data sets (see examples for six species in Hill, 1988; Table 2) indicated whole brain ChE activity was reproducible and associated variance was acceptable for diagnostic purposes in spite of undefined inconsistencies in storage and processing techniques. Perhaps the single most important step in interlaboratory data sharing would be the availability of a ChE

standard for use in quality assurance and interpolation. Such a standard could correct for certain problems associated with inconsistent methods of storage, processing and assay techniques, but the assay method should remain the prerogative of the respective institution.

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Ellwood F. Hill, U.S. Fish and Wildlife Service, Patuxent Wildlife Research Center, Laurel, Maryland 20708, USA.