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Report of the SAB/SAP Joint Study Group on Cholinesterase

Review of Cholinesterase Inhibition And Its Effects

A SCIENCE ADVISORY BOARD REPORT May, 1990

U. S. ENVIRONMENTAL PROTECTION AGENCY

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UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

EPA-SAB-EC-90-014

May 23, 1990

OFFICE OF
THE ADMINISTRATOR

Honorable William K. Reilly
Administrator
U.S. Environmental Protection Agency
401 M Street, S.W.
Washington, D.C. 20460

Subject: Joint Science Advisory Board/Scientific Advisory Panel's review of Cholinesterase Inhibition and Its Effects

Dear Mr. Reilly:

Inhibition of cholinesterase enzyme activity is a mechanism by which an important class of insecticides exerts its effects. Compounds of this class exert toxic effects in mammals, including humans. Because of their widespread application in agriculture they arouse concerns about hazards to agricultural workers, and to consumers as well, who may be exposed to residues in or on agricultural products. EPA is responsible for setting standards for general population exposure to cholinesterase (ChE) inhibitors. To do so, it must evaluate several risk assessment issues whose resolution poses numerous complex and difficult questions.

Agency scientists and consultants have assembled and surveyed the available information and offered a set of provisional recommendations. The Science Advisory Board (SAB) and the Scientific Advisory Panel (SAP) were asked to review these recommendations, which addressed the major issues confronting the Agency. The SAB/SAP Joint Study Group was formed, and subsequently met on September 27, 1989 in Crystal City, Virginia where it was briefed by Agency staff and received comments from members of the public.

The Joint Study Group wishes to recognize the immense amount of work devoted to this effort by the Agency and commends EPA for the clarity with which it has characterized the core issues. Although we feel that a clear and full resolution of these issues can not be accomplished at this time, we welcome the opportunity

to offer our comments on, and to extend the recommendations of, the original EPA Risk Assessment Forum Technical Panel, which reviewed and discussed these issues in a 1988 report, Cholinesterase Inhibition as an Indicator of Adverse Toxicological Effect.

The issues posed to the Joint Study Group and the Group's responses are:

1. Is ChE inhibition (ChEI) in blood (plasma and/or erythrocytes) or brain an adverse effect?

The Joint Group expressed doubt about the validity of plasma and red blood cell (RBC) cholinesterase inhibition (ChEI) as indicators of toxicity. Members pointed out that these measures could not be correlated with recognized adverse effects. In fact, such measures may indicate that the organism's defenses against toxicity are intact.

2. What are the appropriate uncertainty factors for estimating reference doses (RfDs)?

The Group did not propose appropriate uncertainty factors (UF); rather it proposed an improvement over the current approach. The improvement called for fitting a tolerance distribution model to the ChEI-dose response data to determine the lower confidence level of the dose at which the change in ChEI level is just statistically significant, and to adjust that dose level with the UF. The UF should be chosen with the understanding that the lower confidence level dose already accounts, in some degree, for intraspecies variations.

3. Is the developing organism at special risk?

The Group agrees with the Technical Panel conclusion that the evidence for enhanced susceptibility is ambiguous.

4. Can ChEI be considered a valid biomarker of exposure?

The Group expressed unanimity that ChEI is a biomarker for exposure and, whether in blood or nervous system tissue, indicates absorption of the enzyme-inhibiting agent.

5. Related issues involving quantitative models, inter-species differences, neurobehavioral measures, and statistical variability.

The Group concluded that for interspecies predictions, rats appear to be no less accurate than dogs. The crucial element in extrapolation to humans is the similarity in processes determining ChEI levels; these, in turn, depend upon many interacting processes. Complete duplication of human processes in other species is unlikely. Models and other issues are discussed in the report.

The Joint Study Group recommends research to enhance the basis for risk evaluation. The research should include exploitation of currently available data, work on techniques for detecting organophosphate exposure, study of the neurological consequences of ChEI, structure-activity studies, and should address ecological concerns and other subjects noted in the report.

We appreciate the opportunity to review these issues, and look forward to your response.

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Scientific Advisory Panel

Dr. Raymond Loehr, Chairman
Science Advisory Board

Dr. Bernard Weiss, Chairman
Joint SAB/SAP Study Group

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1.0 Executive Summary Inhibition of cholinesterase enzyme activity is a mechanism by which an important class of insecticides exerts its effects. Compounds of this class exert toxic effects in mammals, including humans. Because of their widespread application in agriculture they arouse health concerns, not only about exposure of agricultural workers, but also about exposure of consumers who may be exposed to minute residues in or on agricultural products.

EPA is responsible for setting standards for general population exposure to cholinesterase (ChE) inhibitors. To do so, it must evaluate several risk assessment issues whose resolution poses numerous complex and difficult questions. Many of the difficulties stem from the lack of crucial data, so that answers to those questions necessarily are based on scientific judgment. Agency scientists and consultants have assembled and surveyed the available information and offered a set of provisional recommendations. The Science Advisory Board/Scientific Advisory Panel (SAB/SAP) Joint Study Group was asked to review these recommendations, which were framed to respond to the major issues confronting the Agency. The Joint Study Group recognizes the immense amount of work devoted to this effort and commends EPA for the clarity with which it has characterized the core issues. Although we feel that a clear and full resolution of these issues cannot be accomplished at this time, we welcomed the opportunity to offer our comments on, and to extend the recommendations of the original EPA Risk Assessment Forum Technical Panel, which reviewed and discussed these issues in a 1988 report, Cholinesterase Inhibition as an Indicator of Adverse Toxicological Effect.

The issues posed to the Joint Study Group were described as follows:

a) Is ChE inhibition (ChEI) in blood (plasma and/or erythrocytes) or brain an adverse effect?

b) What are the appropriate uncertainty factors for estimating reference doses (RfDs)?

c) Is the developing organism at special risk?

d) Can ChEI be considered a valid biomarker of exposure?

e) How should the following related issues be treated?

1. Other quantitative models to assess dose-response

2. Species differences and surrogates for humans

3. Neurobehavioral measures of ChEI

4. Appropriate test methods for ChEI

5. Statistical Procedures pertinent to variability

The Joint Study Group found several of the issues, as posed, almost inseparable, so that its responses to each individually also bear upon the others.

First, the Group expressed doubt about the validity of plasma and red blood cell (RBC) ChEI as indicators of toxicity. In addressing the issue of vulnerability of the developing organism, we note that, although maternal exposure to ChEI agents may also expose the fetus, and alter brain ChE levels, the consequences for nervous system development and postnatal function have received no more than minimal study. The Group agrees with the Technical Panel conclusion that the evidence for enhanced developmental susceptibility is ambiguous. The Joint Study Group expressed unanimity that ChEI is a biomarker for exposure, and, whether in blood or nervous tissue, indicates absorption. The relationship between degree of ChEI and toxicity remains unclear, and correlations between exposure indices and neurotoxic manifestations tend to be weak.

2.0 **Background** Over the past several years, the Agency has sought to develop and implement risk assessment methodologies through a consensus-building process reflecting participation by scientists in all parts of the Agency, and to apply these methodologies in a consistent manner across all EPA program offices and regions. The primary institutional vehicles to accomplish this goal are the Risk Assessment Forum, administratively housed in the Office of Research and Development, and the Risk Assessment Council, an assembly of senior EPA managers chaired by the Deputy Administrator of the Agency.

Both of these organizations have been concerned with issues relating to cholinesterase inhibition, since it seems to represent an important endpoint for assessing exposure and may have potential for assessing health effects of certain types of agents (e.g., monitoring the exposure of human populations to organophosphate pesticides). Measurements of blood cholinesterase are conducted as a surrogate for the observation of changes occurring in the central and peripheral somatic and autonomic nervous systems. This is necessary because of the technical difficulties inherent in attempting to measure directly these effects in tissues innervated by cholinergic neurons. Exposure to ChE inhibiting agents can be inferred from blood ChEI because most such compounds cause an irreversible inhibition with only an extremely slow reactivation.

Enzyme inhibition by most carbamates is so readily reversible that its usefulness as an indicator of absorption of this pesticide class is dubious except under controlled experimental conditions. Some carbamates and thiocarbamates, however, can produce ChEI evoking clinical manifestations similar to those produced by OPs.

Interpretation of the biological significance of cholinesterase inhibition and its use in assessing human risk has been hampered by the lack of a consensus as to what level of which kind of cholinesterase inhibition (i.e. plasma, RBC, brain) is associated with overt toxicity. Extensive cholinesterase inhibition may be observed in the absence of any distinct signs of overt toxicity. A critical or "threshold" level of enzyme inhibition below which there is no biologically relevant or truly adverse health effect has not been established for any of the possible loci cited above. This may, in part, be attributable to our insufficient knowledge about the effects of cholinesterase inhibition on various neurotransmitter or other physiological systems or it may be a consequence of biological variability. Use of animal data and uncertainties associated with cross-species extrapolation pose additional problems in assessing human risk.

Because there were several organizations within the Agency developing risk assessment and/or regulatory positions on substances shown to produce cholinesterase inhibition (ChEI), a Technical Panel was formed in 1988, under the auspices of the Risk Assessment Forum, to address the relevant issues and provide a consistent approach. The Technical Panel was charged with

reviewing the relevant literature and preparing a report which would identify the appropriate uncertainty factor(s) to employ in the quantitative assessment of this endpoint (e.g. the derivation of a Reference Dose) and with assessing the consequences to the developing organism of pre- and perinatal exposure to cholinesterase inhibitors.

3.0 Charge to The SAB/SAP Joint Review Group The Forum Technical Panel, and a subsequent Colloquium, made a number of specific recommendations and developed a set of "working principles" which can be applied to the interpretation and use of cholinesterase data. These "working principles" are outlined below. The SAB/SAP Joint Panel was asked to evaluate the scientific bases for these principles and their relevance to assessing human risk. The Risk Assessment Forum and the Risk Assessment Council were particularly interested in the Joint Group's views on four major issues which devolve from the Technical Panel report and are discussed below. In addition, two other issues, not specifically addressed in the Technical Panel report were also posed to the Joint Study Group for consideration. The issues follow below:

3.1 Interpretation Of Cholinesterase Inhibition: An Adverse Effect? A major focus of the Technical Panel report reviewed by the Joint Study Group was on the interpretation of ChE inhibition and its relevance to adverse outcomes. The Panel reached the following conclusions:

a) Although correlation of CHE inhibition with the nature and severity of an overt response is difficult to predict and is dependent upon many variables, statistically significant inhibition of cholinesterase (i.e., plasma, RBC, or CNS) is usually considered a potentially adverse effect.

b) Statistically significant inhibition of cholinesterase in the CNS should always be considered an adverse effect.

c) Statistically significant plasma or RBC cholinesterase inhibition should be considered biologically significant unless an exception can be made on a case-by-case basis as reflected by such factors as dose-response relationships, comparative pharmacokinetics and elements of study design.

The Joint Panel was asked to comment on the validity of the recommendations and findings above, and suggest any revisions it thought appropriate.

3.2 Recommended Uncertainty Factors For RfD Estimation The Technical Panel recommended use of the following uncertainty factors for estimation of RfDs:

a) A factor of 10 for a NOAEL (No Observed Adverse Effects Level) based upon in vivo human RBC AChE or plasma BuChE data.

b) A factor of 100 for a NOAEL based upon animal brain AChE, RBC AChE, or plasma BuChE data.

The SAB/SAP Study Group was asked to comment on the merits of Recommendations a) and b). In addition, the Panel was invited to comment on interspecies extrapolation, specifically concerning use of the dog, and as to whether or not a 10-fold uncertainty factor is adequate to account for human variability.

3.3 The Developing Organism: A Special Case? Although the consequences of exposure to cholinesterase inhibitors during development remain, in large measure, unknown, the developing organism may be especially vulnerable to the effects of ChE inhibitors. The Technical Panel noted that while some ChE inhibitors can cause alterations in ChE levels in the developing brain and peripheral nervous system, a direct relationship between decreased ChE activity and abnormal development has not been established. The Technical Panel also noted that this does not preclude special concern for the developing organism. The Panel recommended additional research to determine whether an equivalent level of ChE inhibition places the developing organism at risk for more severe effects than would occur in the adult.

The views of the Joint Study Group were sought to assess the merits of the Colloquium Panel's suggestions, given current scientific understanding of the developing nervous system and the potential effects of agents which may inhibit cholinesterase levels.

3.4 Cholinesterase Inhibition: A Biomarker? Cholinesterase

enzymes are marker enzymes which reflect exposure to, and the degree of absorption of, ChE inhibitors and, in addition, may reflect impact on biological systems. ChE inhibition should, therefore, be addressed as a biomarker of both exposure and also as of a potential neurological effect. The SAB/SAP Panel was asked to comment on this issue.

3.5 Additional Issues There were a number of other issues which were not specifically addressed or which received less attention in the Technical Panel Report. The Joint Group was asked to comment on the following questions:

a) Are there other quantitative approaches (e.g., biologically based or statistical models) which can provide a better assessment of dose response than the RfD?

b) If the RfD method is used, are there specific classes of agents to which the dog (or other species such as the non-human primate) may be more sensitive and therefore warrant a reduced uncertainty factor (e.g, 10)?

c) Are there other neurobehavioral measures which have been found to be sensitive and reliable measures of effects associated with cholinesterase inhibition?

d) If the operational criteria for adversity is statistical inhibition of ChE, study conduct will be of critical importance. Is there sufficient scientific consensus as to the most appropriate test methods to utilize?

e) Given the variability of cholinesterase levels observed even in the absence of exposure to cholinesterase inhibitors, are there statistical procedures that the Study Group would recommend to deal with this problem?

3.6 Research Needs The Technical Panel identified a number of research needs. Comment was invited on the merits of the identified research needs; identification of appropriate additional areas of research was also requested.

4.0 Detailed Findings This report is not a detailed review of the available literature, but a response to the recommendations of the

Technical Panel. Literature citations are provided sparingly, and only to amplify certain singular points.

4.1 ChEI As An Adverse effect The relevant questions addressed here are: Is a "statistically significant" reduction in ChE a criterion for establishing a NOEL or NOAEL?; Is a reduction of brain ChE, without exception, an adverse effect?; What role should be played by reversibility? Although the Technical Panel recommended reliance on statistical significance, it also noted that it is difficult to predict the correlation of ChEI with the nature and severity of an overt response which depends on many variables.

The Group expressed doubt about the validity of plasma and red blood cell (RBC) ChEI as indicators of toxicity. Members pointed out that these measures could not be correlated with recognized adverse effects. In fact, such measures may indicate that the organism's defenses against toxicity are intact; perhaps this is the reason blood enzymes sometimes can be severely depressed in the absence of signs of poisoning. Another explanation for the apparent lack of toxic signs, despite substantial ChEI, is the high turnover rate for AChE, amounting to 300,000 moles/minute for ACh. Under such circumstances, a high level of inhibition may not markedly influence the rate of ACh hydrolysis. In addition, the body contains many esterases, typically in abundance, offering many possibilities for interaction without adverse effects. It is difficult to argue, moreover, that reduced brain ChE, given the widespread distribution of ChEs, is adverse in itself although it should be taken more seriously than ChEI in blood. Even so, the large functional reserve in brain provides an intrinsic protective mechanism, and compensatory processes are also operative. Receptor populations undergo up- and down-regulation, and normal behavior can occur in animals concurrently experiencing marked brain ChEI.

If statistically significant (or, alternatively, 20%) ChEI is lacking in cogency and validity as a measure of toxicity, what alternatives might be pursued? The one making the most scientific sense is to define toxicity on the basis of functional (e.g., behavioral, electrophysiological) or morphological indices. Then, proceed to determine what degree of ChEI, in a critical tissue, predicts the neurotoxic response, and set the exposure level accordingly. Such an approach is common in toxicology.

Reliance on statistical significance is a separate issue to be discussed later. Note here that Group members expressed considerable skepticism about the validity of such an approach.

4.2 Uncertainty Factors for Calculating RfDs This question divides into many ramifications, some of them deriving from current Agency practices. In essence, the Technical Panel proposed to compute a NOAEL or NOEL on the basis of statistically significant ChEI (blood or brain), then apply a specified uncertainty factor (UF) large enough to include a NOAEL derived from the most sensitive members of the human population. The question posed in section 4.1 above, according to the majority of the Joint Group, is premature because we view the "statistically significant" criterion as seriously flawed. The objections arise from two problems; first, with the intrinsic statistical assumptions; and second, as described above, the absence of a firm criterion founded in toxicology.

Risk assessment is acknowledged as the basis for EPA actions and initiatives. The approach advocated by the Technical Panel, however, is not based on risk estimation. It is, instead, a convenient management tool. It does not incorporate the fundamental measure of toxicology, the dose-consequence relationship. It does not take into account statistical power, dose spacing, variability, or trends. Most notably, it is an arbitrary captive of the sensitivity of a particular method and its variability. As the SAB has noted repeatedly, the established NOAEL/UF approach may reward low sensitivity and expanded variability because these properties lead to inflated NOAELs.

For example, an experiment with a small number of animals in each dose group might yield a NOAEL significantly higher than a LOAEL based on an experiment with a larger number of animals in each dose group. If ChEI variability (which seems high in many circumstances) is broadened even more by aberrant observations, additional NOAEL inflation may result.

The most attractive alternative to the current approach would involve the estimation of a dose-response surface that relates exposure to both incidence and severity of nerotoxicity, and that, given appropriate tolerance distribution models and a specified ChEI level, would yield actual estimates of the risk of adverse

effects. Unfortunately, available information and data do not currently allow such an approach, however. An improvement over the current approach however, would be to estimate a dose-activity relationship by fitting a dose-tolerance model such as the probit model or expressions such as $y = \hat{a} + \hat{b}x$ or $y = \hat{a} + \hat{b}\log x$, or another function, where x = dose or exposure, and y = ChEI activity level in selected tissues such as blood and brain, if these reasonably approximate the probit model over the range of extrapolation. For estimated values of a and b, determine the value for x at which the value of y becomes significantly different from zero, or is associated with 20% ChEI in blood. The latter (20% ChEI) is most closely associated with the current approach, the former with the proposed approach. The application of UFs to this dose level, which takes into account the total dose-response data, can be combined with statistical power calculations. The UF can be applied to the lower statistical confidence limit of the estimate of dose (associated with a statistically significant change in activity level or with a 20% ChEI) to penalize small samples or large variability. Or the UF could be applied to the dose inducing any alternate response, such as, say, 30% ChEI.

Even such statistically attractive options founder without cogent toxicological endpoints, as noted earlier. The basis for statistical modeling should be appropriate functional measures based on both the central and peripheral nervous systems. Markers of exposure (see 4.4 below), even with the current risk assessment protocol, are interpretable only with parallel indicators of toxicity. Using one index to serve as both a measure of exposure and a measure of toxicity poses problems that are highlighted by the risk assessment process.

The appropriate UFs, then, have to be seen in that context. Large UFs seem generally unwarranted because of inherent margins of safety between ChEI and detectable neurotoxicity; that is, substantial ChEI may be measured in the absence of detectably adverse functional or morphological effects. However, given our elementary grasp of the correlations between measures of ChEI in blood and neurotoxicity, we should be wary from another direction about how we apply UFs; they are not applied to the appropriate endpoint. We need to define more direct endpoints. Behavioral measures, for example, may offer useful indicators because they can be used for monitoring adverse effects and, also, dispense with the intervening, indirect variable of ChEI. With both neurotoxicity

and ChEI measures available concurrently, together with dose-response (effect) information, the Agency can proceed to apply UFs on a much more rational basis.

New experiments on adult humans, with proper safeguards, and a thorough review of the available data, might be considered for some purposes. Volunteers are used by some manufacturers, and of course, Food and Drug Administration protocols make provisions for observations in humans during drug development. The doses chosen, of course, would be very low, but could provide guidance about the dose sufficient to inhibit ChE in blood. Such observations, however, would provide minimal information about possible chronic effects and potential hazard to the fetus.

4.3 Vulnerability of The developing Organism to ChEI Agents

For many environmental agents, the developing brain offers an especially vulnerable target. Lead, methylmercury, ethanol, and PCBs are among the agents identified as posing enhanced risks to the developing brain. Not only are nervous system elements undergoing rapid growth, an important dimension of vulnerability, but the immature blood-brain barrier permits entrance of agents that ordinarily would be excluded.Also, the drug-metabolizing enzymes that later function to detoxify many chemicals are not yet available. At the same time, this biochemical immaturity could also afford some protection against OPs such as the phoshorothionate insecticides. These compounds represent a subclass that requires metabolic activation by cytochrome P-450 dependent monooxygenases for conversion to the final anti-ChE metabolite.

For other agents inhibiting ChE, the available information is less compelling. Although maternal exposure to ChEI agents may also expose the fetus, and alter brain ChE levels, the consequences for nervous system development and postnatal function have received no more than minimal study. Even the comparative sensitivity of fetal and adult brain, as measured by ChEI, is the subject of conflicting findings. But conflicts would be the predicted outcome of assessments that differ in agent, dose, and timing of exposure and stage of development. The Group agrees with the Technical Panel conclusion that the evidence for enhanced developmental susceptibility is ambiguous.

The Joint Study Group, however, believes that the Agency

should also consider the evidence implicating advanced age as a period of enhanced vulnerability to ChEI agents. Research indicates, for example, that older rats exhibit more pronounced toxic syndromes than young rats. "Developmental toxicity" should not be confined to early development, especially given the incidence of neurodegenerative disorders in our aging population. A more scientifically rigorous point of view is that variations in susceptibility occur through the total life-span.

4.4 ChEI As A Valid Biological Marker of Exposure The Joint Study Group expressed unanimity on the answer to this question, and agreed that ChEI provides an index of exposure, or more specifically, absorption. In fact, it is exactly the lack of correspondence between exposure markers and neurotoxic measures that fosters the Group's skepticism about collapsing them into a single index. That is, ChEI, whether in blood or in nervous tissue, indicates exposure rather than toxicity. The amount of observable inhibition, in fact, could just as well be interpreted as a measure of detoxification efficiency, to the extent that it reflects scavenging and inactivation of the agent. Although, with some carbamates, substantial ChEI and serious toxicity may occur concurrently, with others, the rapid reactivation of ChE makes ChEI less useful as a measure of exposure. Reactivation, however, is a process that depends on both rate of inhibition and rate of recovery. With these agents, also, plasma ChE activity may be reduced to undetectable levels without causing lethal outcomes, or even signs of poisoning.

In accepting the validity of ChEI as a measure of exposure, the Group also remains cautious about direct quantitative interpretation. Plasma (butyryl) ChE assays are not standardized, but, instead, are conducted with a variety of methods, leading to a highly variable literature. Perhaps standardization could be expedited by adopting commercial kits for spectrophotometric assays. However, because most contain proprietary buffers, their constituents cannot be reproduced and tested in research laboratories. Also, for detection of exposure at environmental levels, if such data are deemed necessary, High Pressure Liquid Chromatography (HPLC) and Mass Spectroscopy (MS) may be required. These techniques possess the advantage that they can detect hydrolytic products at OP dose levels insufficient to inhibit ChE. Assays of brain ChE, as noted above, arouse additional questions. Should the various forms, at least those so far identified, be

assayed individually? Should anatomical distribution be considered, and regional brain analyses performed? The SAB/SAP Joint Study Group endorses the need for clearly defined standard procedures for all tissues and views as feasible, even advantageous, a protocol that included several methods, each contributing a different facet of information.

Underlying questions such as these are, again, the undependable connections between blood ChE, brain ChE and neurotoxicity. With some agents, ChEI of 20% in blood may be associated with toxic manifestations. With others 90% ChEI is required. Predictions based on structural chemical features might help in the future, but are still not reliable enough to screen compounds that evoke a unique response. Although more consistent relationships might arise by relying on erythrocyte rather than plasma ChE in assays of many OPs, the measurements are more laborious and difficult. Furthermore, even the correlations between brain ChEI and functional disturbances may be abysmally low within a single experiment, as in the recent report by Jimmerson et al[1], so that generalizations about effects and biochemical lesions are of limited accuracy. In essence, then, ChEI serves primarily as a biomarker of exposure, not of neurotoxicity; the latter is defined independently.

4.5 Related Issues Most categories of what were classified as related issues have already been discussed. Species variations remain a fulcrum of debate. They were addressed by the Joint Study Group and by the Technical Panel from the standpoint of a suitable surrogate species for humans. At least in the past, some investigators have advocated the dog as providing the closest correspondence with humans. Few data support such a position, even though dog data are routinely collected for pesticides. For interspecies predictions, however, rats appear no less accurate (nor more innacurate) than dogs. The crucial element in extrapolation to humans is the similarity in processes determining ChE levels and these, in turn, depend upon many interacting processes. They include bioactivation and degradation of liver enzymes, breakdown by plasma enzymes, and absorption and distribution parameters. It is unlikely that any combination of such processes found in the human body will be duplicated

[1]Toxicology 57:241-254, 1989

completely in any other species.

The questions of quantitative models to assess dose response, and statistical procedures pertinent to variability were included in the discussion of UFs; appropriate test methods for ChEI were discussed above with section 3.4; and the issue of neurobebavioral methods will be discussed under Research Needs and Recommendations (sections 3.6 and 3.7 below).

4.6 Research Needs Risk assessment is ultimately an extension of, and an extrapolation from, scientific data. Because both inflated and inadequate risk estimates engender costs to society, research to enhance the basis for risk evaluation is a sound investment. The recommendations for research support listed below are designed to support such an enhancement. They are not intended to delay actions by the Agency on the questions posed to the Joint Study Group. The Group recognizes that perfection in regulatory decisions will always remain an elusive target.

4.6.1 Exploitation of Currently Available Data Existing data on correlations between ChEI and manifestations of toxicity should be examined more thoroughly. An enormous amount of information, especially on humans, may be available from classified military files that probably could safely be declassified. The ChE data base generated by industry research, although unavailable to the general public, could also be surveyed by EPA scientists, as could the Agency's own data from study populations such as applicators, formulators, and others. Both sources of information would amplify, for example, the relative contributions of intra- and interindividual variability.

4.6.2 Detection of OP Exposure New techniques for detecting OP exposure are under investigation, e.g., transdermal patches permitting the analysis of parent OP compounds and their metabolic products in tissue fluids. An expansion of this research, which could also profit from making use of exposed agricultural workers, would directly benefit the Agency. Leukocytes and other blood elements contain a variety of neurotransmitter receptors, including those mediating cholinergic function. Receptor assays based on these blood cells might provide another means of detecting antiChE exposure. Direct measures of ChEI, however, remain the most dependable estimates.

4.6.3 Neurobehavioral Consequences of ChE Inhibition Although the neurobehavioral consequences of ChEI have been the focus of many publications, immense gaps in knowledge remain. These take several forms:

a) We possess inadequate information about the long-term consequences of exposure, especially at levels insufficient to elicit overt signs of poisoning. For example, are there subtle adverse effects associated with chronic exposure that are not severe enough to induce clinical signs? Some studies indicate that psychological testing can uncover such corollaries[2]. Are there forms of what has been called silent toxicity, such as aberrant responses to certain drugs? Published data suggest this to be an important question[3]. Are there effects remote in time from early developmental exposure? Findings based upon many other classes of toxicants and drugs underscore the need to explore this question in comprehensive fashion.

b) To what degree is acute poisoning reversible? Is there subtle neurobehavioral impairment that lingers once ChE levels return to normal and the acute symptoms subside? In part, such a question arises from observations of workers exposed in the past to high levels of OPs during accidents in the manufacture or field application of such agents for chemical warfare[4], and in part from animal studies documenting lesions following exposure. For both chronic exposures and acute aftermaths, a detailed series of laboratory investigations on processes such as memory, because of their connection with cholinergic neurotransmission, is warranted. A recent paper by Savage et al, which noted impaired psychological test performance long after acute poisoning episodes, supports the need to ask such questions[5].

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[2]Levin et al, Arch. Gen. Psychiat. 33:225, 1976

[3]See Bignami et al, Fund. Appl. Toxicol. 5:S213-S224, 1985

[4]Duffy et al, Neurotoxicology 1:667, 1980; Whorton and Obrinsky, J. Toxicol. Environ. Health 11:347, 1983.

[5]Arch. Environ. Health 43:38-45, 1988

(The next two items not only embody research needs, but are also important components of standard setting.)

c) Given the lengthy history of anti-ChE agents, the lack of information on the correlation between central and peripheral nervous system effects and ChEI is surprising. For example, are the temporal properties similar? Might peripheral nervous system ChEI and associated functional measures better reflect acute toxicity?

d) Cholinergic mechanisms in the brain do not act in isolation. Numerous interactions between cholinergic and other neurotransmitter and neuromodulator systems are now recognized, as is the coupling of receptors with second messengers and other steps in the chain of events by which neurotransmission occurs. How do these entwined mechanisms modify or control the action of anti-ChE agents?

e) Measurement of whole-brain ChE, especially given the many varieties of esterases present in brain, could prove misleading and, in addition, represent one of the sources of the low correlations with functional endpoints. Regional analyses, based upon biochemical measures, receptor assays, and, possibly, histochemistry, when correlated with specific functional endpoints, could help resolve several puzzling discrepancies. Such research would also help clarify the contributions of various cholinergic pathways in the brain whose functional roles are not well understood.

4.6.4 Sources of Individual Variability The causes of variability, although mostly obscure at present, surely arise, in part, from genetic predispositions. At least one phenotypic variant of butyrlChE has been identified that increases vulnerability to antiChE agents. The defective gene underwent a 100-fold amplification in a farmer chronically exposed to an OP insecticide[6]. Current techniques of molecular biology can be used to identify other variants whose bearers may be at greater risk.

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[6]See Prody et al, PNAS 86:690-694, 1989

4.6.5 Temporal Focus for Studies The total life cycle, not just early development, should be examined for relative vulnerability to ChEI and its consequences, especially because of the association between cholinergic function and certain facets of nerodegenerative disease.

4.6.6 Ecological Concerns Given the growing concern within the Agency over ecological consequences and ecological risk assessments, the impact of antiChE agents on wild populations requires careful study. One example: minimally impaired alertness or motor function in a specific wild animal population might result in greater losses to predators or to accidents, and alter a large range of ecological inter-relationships and balances.

4.6.7 Structure-activity Studies These studies, although expensive, should be expanded in conjunction with the other research needs described above.

5.0 Recommendations The Joint Study Group is distinctly aware of EPA's responsibilities and the dilemmas posed by lacunae in those data sets upon which reasonable risk reduction policies must be founded. The Group also recognizes EPA's eagerness to move beyond current, often improvisational practices. At the same time, the members of the Group remain unconvinced by the Technical Panel's arguments for changing these current practices, imperfect as they are, and substituting another batch of equally unsatisfactory criteria.

Four recommendations addressing this situation arise from the Joint Study Group's deliberations:

a) Base the criteria for adverse effects upon adverse effects. That is, define an adverse effect on the basis of functional (behavioral, electrophysiological) measures, accompanied, where feasible, by morphological indices such as those provided by both newer and established histochemical techniques. Determine the associated biochemical indices (plasma, RBC ChEI), and, for certain animal studies, brain ChEI values. Include a range of exposure levels to encompass minimal to marked neurotoxicity and preserve individual organism data to facilitate statistical analysis. An ironic counterpoint to the Joint Study Group agenda was the meeting, on the following day, devoted to the OPP/FIFRA Guidelines for

Neurotoxicity. Such criteria should be implemented quickly and a research program adopted to insure continued improvement in sensitivity and specificity.

b) Replace the NOAEL/UF strategy with one based on the kinds of dose-consequence data available from the information in (1) above. Use the exposure data (such as plasma or RBC ChEI) in company with the performance data to construct a specified function derived from a recognized tolerance distribution, or, better yet, a dose-response correlation surface (See Section 4.2). From these, distill a specified level of ChEI based upon, say, a 10% decrement of performance. To the 95% lower bound, attach a UF to yield the RfD.

c) Reexamine structure-activity relationships on the basis of the kinds of information sought in (1) above. A more complete grasp of toxicity should permit higher correlations between structural features and potential toxicity.

d) Devote more support to peripheral nervous systems assessments; although it poses some difficult technical problems, using such assessments, based on correlations of ChEI and functional measures, may offer an improved basis for setting exposure standards.

Such an approach as is outlined above is also some distance from what might be regarded as optimal, and also requires scientific judgement because of variations in pharmacokinetic parameters, metabolic transformation, and other associated factors. It comes closer, however, to scientific rigor than what so far has been implemented. It may also be more demanding of both industry and EPA resources than the current practices, but, given how much both have invested because of other uncertainties, that assertion could he argued. Finally, it is laboratory rather than debate intensive, so that it is more likely to nurture improved practices than what in essence is a minor change in the status quo and that does not seem to the Joint Study Group to offer a significant advance in the protection of public health.