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Interpretation – Evaluation of the Neurotoxic Potential of Chemicals in Animals

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ECETOC Special Report No. 6

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PREFACE

This report has been prepared by ECETOC for use by a Special Expert Group of DG V who are expected shortly to examine the neurotoxic potential of solvent chemicals in the context of their Indicative Limit Value programme. The report is based on ECETOC Monograph No. 18 "Evaluation of the Neurotoxic Potential of Chemicals".

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KEY DOCUMENT TO ASSIST IN THE INTERPRETATION AND EVALUATION OF THE NEUROTOXIC POTENTIAL OF CHEMICALS IN ANIMALS

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INTRODUCTION

The ultimate objective of animal toxicity studies is to provide information that will enable the safe use of the chemical through hazard identification, hazard assessment and risk assessment. This document provides a scientific view on how to effectively evaluate the neurotoxic potential of chemicals in laboratory animals. A more detailed monograph on this subject is available from the European Centre for Ecotoxicology and Toxicology of Chemicals (ECETOC, 1992).

Toxicity tests in laboratory animals are a major component of hazard identification, hazard assessment and risk assessment. Standard toxicity studies with rats, mice, rabbits and dogs provide many opportunities to identify target organs and treatment-related effects in all organ systems including the nervous system. Thus, a significant amount of neurotoxicity data can be obtained from standard toxicity studies.

Neurotoxicology is a relatively new branch of toxicology and relies on a number of disciplines including psychology, neurology, physiology, pharmacology, biochemistry and pathology. Each discipline has a range of methods, with varying complexity, specificity and applicability for investigating neurotoxic effects of chemicals.

Potential damage to the nervous system is difficult to assess because of wide variations in normal function of the nervous system as well as its plasticity, residual capacity and compensatory mechanisms (WHO, 1986). Furthermore, there may be difficulties with the interpretation and assessment of the relevance of the results from certain tests. Essentially all substances could be classified as "neurotoxic" since all chemicals are toxic at some dose level and will induce some behavioural change at these dose levels. Therefore, detection of those

compounds that may cause direct, persistent, adverse effects on the nervous system should be given the most critical attention in order to protect human health in the workplace and the environment.

Evaluation of the neurotoxic potential of a chemical should include descriptions of functional and morphologic effects as well as the determination of the dose response, time course, reversibility of effects and the NOEL. Differentiation between direct and indirect neurotoxic effects of a substance is critical for scientific and regulatory considerations. Also, investigation of species specificity, toxicokinetics and the mechanism of a neurotoxic effect may provide useful information for the risk assessment process.

UNDERSTANDING AND DEFINING NEUROTOXICITY

A simplistic definition of neurotoxicity (or a neurotoxic effect) is an adverse change in the structure or function of the nervous system that results from exposure to a chemical substance. A recent publication (OTA, 1990) cautions that the definition of neurotoxicity "hinges on interpretation of the word 'adverse,' and there is disagreement among scientists as to what constitutes 'adverse change.'"

Interpretation of the results from neurotoxicity studies, in terms of potential human health effects, requires an understanding of several significant issues:

- What constitutes an adverse effect on the nervous system?

- What distinctions should be made between transient effects and persistent effects?

- What is the significance of direct as opposed to indirect effects on the nervous system?

- When should a compound be considered a neurotoxic substance?

ADVERSE EFFECTS

A judgment as to what constitutes an adverse effect on the nervous system depends primarily on the nature of the change (morphological, neurochemical, neurological, or behavioural), the degree of change and whether the effect is transitory or persistent.

A reasonable definition of **an adverse effect is any treatment-related change which interferes with normal function and compromises adaptation to the environment.**

The term "adverse" should suggest the concept of disease and implies that the change interferes with normal function (e.g., the change is maladaptive to the individual). Adverse effects should be considered only in a toxicological sense rather than as any unwanted effect. Effects without recognised maladaptive consequences may have beneficial, indifferent or unknown consequences, but are not necessarily adverse.

Most morphological changes such as neuronopathy, axonopathy or myelinopathy would be considered adverse even if the changes were mild and/or transitory. On the other hand, transitory hypertrophy of astrocytes could be viewed as an adaptive, physiological response. A more complicated example is the transitory reduction in the number of dendritic spines on pyramidal neurons in rats exposed *in utero* to ethanol (Ferrer et al, 1988). Although a reduction in the number of dendritic spines in 15-day old rats was followed by morphological recovery at 90 days of age, the results would not necessarily prove functional recovery.

Neurochemical changes in the nervous system of experimental animals should not necessarily be interpreted as adverse without additional correlative information. For example, gerbils exposed to near-lethal levels of dichloromethane (DCM) for 2 months and examined 4 months post-exposure, were reported to have changes in brain levels of DNA as well as glial fibrillary acidic protein (GFAP) and S-100 protein (Rosengren et al, 1986). A subsequent study was conducted in order to determine if exposure to DCM was associated with any toxicological adverse effects on the nervous system (Mattsson et al, 1990a). Detailed clinical, neurofunctional and neuropathological examinations of rats exposed to high levels of DCM for 13 weeks did not reveal any persistent post-exposure, treatment-related effects and call into question the relevance of the changes reported in the initial study.

Although neurotypic and gliotypic proteins have been proposed as biochemical markers of neurotoxicity (O'Callaghan, 1988), the EPA Science Advisory Panel concluded that radio-immunoassay measurement of GFAP in the brain is not sufficiently validated as an indicator of neurotoxicity to be incorporated in test guidelines (SAP, 1989). Likewise, there is considerable doubt about the validity of plasma and red cell cholinesterase inhibition as an indicator of neurotoxicity (SAB/SAP, 1990) since these biochemical measures are not correlated with recognised adverse effects. The Panel recommended that adverse effects on the nervous system should be defined on the basis of functional measures (behavioural, electrophysiological) accompanied by morphological indices such as histochemical techniques.

TRANSIENT VERSUS PERSISTENT EFFECTS

Transient, acute neurological effects of compounds should be distinguished from permanent or chronic effects. Many chemicals can have non-specific

pharmacological effects on the nervous system at high exposure levels, but these effects are not relevant for prediction of neurotoxic effects at occupational or environmental exposure levels.

The potential consequences of neuropharmacologic effects vary significantly. Similar to the situation with anaesthetics, some reversible functional or behavioural changes that are not associated with permanent morphological alterations would not be considered adverse in themselves. On the other hand, pharmacologic effects in humans may induce adverse consequences by a reduction in vigilance which possibly could result in accidents. Furthermore, chemicals such as organophosphates clearly have adverse acute neurotoxic effects because of their pharmacologic mechanism of action.

Dichloromethane (DCM) is an example of a substance with transient neuropharmacological effects, but no confirmed neurotoxic effects (Mattsson et al, 1990a). DCM causes central nervous system depression at relatively high concentrations and also alters neurophysiological functions of rats during exposure to 2000-5000 ppm DCM. Post-exposure functional tests (observational battery, grip strength, and evoked potentials) along with detailed pathological examinations of rats exposed to as much as 2000 ppm DCM for 6 h/d, 5 d/wk for 13 weeks did not reveal any treatment-related changes. Thus, even though DCM does have sedative and anaesthetic properties at relatively high concentrations, brain injury appears unlikely.

DIRECT VERSUS INDIRECT EFFECTS

Another important concept in the practical definition of neurotoxicity is the significant difference between direct (primary) as opposed to indirect (secondary) effects on the nervous system. A wide variety of disease conditions such as nutritional and metabolic disorders, diabetes mellitus, liver disease and kidney disease may have secondary effects on the function and structure of the nervous system, such as encephalopathy or polyneuropathy (Robbins and Cotran, 1979). Exposures which do not cause primary toxicity in the liver or kidney, for example, would not result in any secondary effects on the nervous system. Thus, differentiation between direct and indirect effects of a substance on the nervous system is critical for scientific and regulatory considerations.

The indirect effect of substances on the nervous system is a major concern for the interpretation of acute toxicity studies which are conducted at very high dose levels. Substances which are not directly toxic to the nervous system can cause signs such as convulsions, tremors or ataxia at toxic or lethal dose levels. Thus, essentially all substances could be classified as "neurotoxic" since all chemicals are toxic at some dose level and will induce some behavioural change at these very high dose levels. These concerns were summarised by the EPA's Science Advisory Panel (SAP, 1989) when they stated "The Panel has serious reservations about the validity of neurotoxicity studies in which the high-dose level results in gross changes which exceed the MTD [maximum tolerated dose] or in which the normal metabolic processes of the body are severely compromised."

The interpretation of neurofunctional data from routine toxicity studies may be confounded by systemic toxicity. Gerber and O'Shaughnessy (1986) evaluated the specificity of several functional and behavioural tests for nervous system toxicity. The results indicated that impairment of organs other than the nervous system as well as reduced food and water intake can mimic the behavioural effects of standard neurotoxic agents. In fact, Gerber and O'Shaughnessy (1986) suggest that "before it can be concluded that a compound is neurotoxic on the basis of behavioural test results, it must be ascertained that non-neural organs have not been damaged by the test compound, and that food and water consumption have not been severely decreased."

Further studies of dietary restriction in rats revealed significant effects on physical, behavioural and neurophysiological parameters after 4 weeks of dietary restriction (Albee et al, 1987). Thus, the relevance of neurofunctional changes is obscure in the presence of general toxicity and in the absence of neuropathological changes (Mattsson et al, 1989).

Interpretation of results from motor activity tests is difficult especially in terms of direct versus indirect effects on the nervous system. A dose-related change in motor activity reflects an effect on the nervous system only in the absence of general toxicity; additional information is required to determine that such an effect actually is adverse (Maurissen and Mattsson, 1989). Conclusions about nervous system involvement based on motor activity can be made only by exclusion and thus, interpretation should be limited to a dose range not associated with general toxicity.

Neurotoxicity should imply a direct effect on the nervous system while behavioural or neurofunctional effects are terms that indicate a more general functional change, whether or not such an effect originates in the nervous system (Maurissen and Mattsson, 1989). Therefore, indirect effects that are detected by neurofunctional tests such as clinical observations, motor activity and other behavioural tests, clearly should be distinguished from direct neurotoxic effects.

DEFINITION OF A NEUROTOXIC SUBSTANCE

Designation of a substance as neurotoxic should be reserved for those xenobiotic compounds or their metabolites that produce adverse effects as a result of direct interaction with the nervous system. Substances which are not directly toxic to the nervous system, but result in neurobehavioural signs as a result of damage to other organ systems, should not be considered as neurotoxicants.

An assessment of potential neurotoxicity should be based on a number of different parameters that are derived from a variety of toxicological tests at relevant dose levels. A combination of functional and morphological tests enhances the ability to discover neurotoxicity.

Inappropriate categorisation of a substance as "neurotoxic" could be best avoided by a clear understanding of the general toxicity of a chemical prior to specialised neurotoxicity studies. Subsequent specialised tests for neurotoxicity should only be conducted at dose levels where no adverse general effects were detected with routine toxicity studies. Thus, a critical point in the evaluation of potential neurotoxicity is the threshold dose for neurotoxicity; substances with neurotoxic effects at levels which are less than the NOEL for other toxic effects would be of concern.

Criteria for categorisation of a substance as a human neurotoxicant were proposed for solvents by Spencer and Schaumburg (1985). Three questions must be answered affirmatively before a solvent is accepted as a human neurotoxicant:

- Does the substance or mixture produce a consistent pattern of neurological dysfunction in man?

- Can this entity be induced in animals under comparable exposure conditions?

- Are there reproducible lesions in the nervous system or special sense organs of exposed human beings and/or animals, and do these abnormalities satisfactorily account for the neurobehavioural dysfunction?

These criteria also are applicable to chemicals other than solvents and would be useful in the categorisation of substances in regard to neurotoxicity for man.

A substance could be considered a 'possible human neurotoxicant' if animal toxicity studies reveal limited evidence of direct, adverse, persistent effects on the nervous system. Indirect effects on the nervous system that clearly are secondary to systemic toxicity would not be a basis for even this category.

When animal neurotoxicity studies provide sufficient evidence of direct, adverse, persistent effects on the nervous system and are likely to be consistent across species, the substance could then be considered as a 'probable human neurotoxicant'.

Finally, fulfillment of criteria similar to those of Spencer and Schaumburg should be applied in order to categorise a substance as a 'known human neurotoxicant'.

METHODS FOR THE EVALUATION OF POTENTIAL NEUROTOXICITY

The design of neurotoxicity studies should incorporate clear objectives and produce interpretable data. The World Health Organisation (WHO, 1986) defined the objectives of neurotoxicity testing as:

- identify whether the nervous system is altered by the toxicant (detection);

- characterise nervous system alterations associated with exposure;

- ascertain whether the nervous system is the primary target for the chemical;

- determine dose- and time-effect relationships in order to establish a no-observed-adverse-effect level.

These objectives translate into a series of questions about the toxicity of a chemical that may be answered with standard toxicity tests as an initial screen and/or more specialised neurotoxicity studies.

STANDARD TOXICITY STUDIES

The term "standard toxicity studies" refers to the toxicity test guidelines of OECD (1981), EEC (1983), EPA/TSCA (1983-84), EPA/FIFRA (1991) and Japan/MAFF (1985). Relevant information concerning potential target organs including the nervous system can be obtained from acute (single dose), subacute, subchronic and chronic toxicity studies and reproduction studies.

Information on Potential Neurotoxicity from Standard Toxicity Studies

Standard toxicity studies are important in the assessment of potential neurotoxicity of a compound because these studies are conducted at relatively high doses, with different duration and routes of administration as well as with several species of animal. Metabolism and pharmacokinetic data often are developed in support of the standard toxicity studies for many compounds. Standard toxicity studies evaluate functional, behavioural and morphological endpoints for the nervous system which may give preliminary or definite indications of the neurotoxicity of xenobiotics (Steinberg, 1987).

The variety of dosing regimens for standard studies is important because some chemicals induce effects after single exposure (e.g., trimethyltin, Hagen et al,

1988; organophosphates, Abou-Donia and Lapadula, 1990) whereas others require repeated exposure (e.g., acrylamide, Bogo et al, 1981). Not only is the duration of exposure important but, for some chemicals, the exposure pattern is important in determining whether or not the material is neurotoxic. For example, exposure to hexane at 1000 ppm 24 h/d, 5 d/wk for 11 weeks produced clear and long-lasting neurotoxicity but exposure at 24,000 or 48,000 ppm for brief (10 minutes) periods 6 or 12 times per day (i.e., equivalent or higher total exposure) produced only slight effects (Pryor et al, 1982).

Contribution from Clinical Observations. Clinical observations included in standard toxicity protocols usually are obtained by cage-side monitoring of animals, as well as during handling at the time of dosing or body weight determination. Clinical observations may indicate changes in motor function (e.g., disturbances of gait, abnormal posture or muscle tone), arousal state (e.g., hyperactivity, apathy or lethargy), psychological state (stereotypes, aggression, biting, licking, self mutilation) or indications of pharmacological effects (sedation, anaesthesia).

Indirect evidence of neurotoxicity from standard toxicity studies may be suggested by the general physiological state of the test animals. The integrity of the autonomic nervous system can be assessed with observations of specific functions such as salivation, lacrimation, urination or defecation.

Clinical observation of adults or pups in standard reproduction studies may give an indication of altered neuromotor functions or arousal states that may be affected by developmental neurotoxicants. Successful mating, delivery and rearing of pups depend on normal behaviour and appropriate function of multiple organ systems including the nervous system. Also, physical and functional landmarks of pups are sensitive parameters of development. Furthermore, reproduction by F1 animals provides additional information on nervous system development.

Contribution from Morphological Examinations. Standard toxicity studies generally include gross examination of most organs and tissues, measurement of the weight of organs including the brain, and histopathological evaluation of brain, spinal cord, peripheral nerve, muscle, eyes as well as many other tissues. Thus, a broad range of cellular elements and functional entities which comprise the nervous system is evaluated by standard toxicity studies.