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Environmental pathology

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Environmental pathology of the nervous system

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The negative public attitude toward environmenta agents

Harmful (adverse) effects on the nervous system can be produced by agents present in food, drinking water, beverages, air and the materials used to provide clothing, housing, entertainment, transportation, disinfection, cleaning, etc.. In contrast to medication, which is deliberately consumed due to its recognized health-improving and life-saving functions despite possible deleterious side effects, the exposure to environmental agents, especially to "unnatural" ones is inadvertent and unwanted. Consumers are not ready to accept any side effects of environmental agents. In general, such environmental agents can be considered chemicals, although with the recent advent of gene technology, differentiating between "chemical" and "nonchemical" agents is increasingly difficult. One could say that the potential effects of genetically engineered living organisms on humans are of microbiological concern, but toxicology cannot shun its responsibility for potentially harmful effects of substances produced by genetic engineering.

The tasks and goals of neurotoxicology

To minimize the exposure to potentially harmful agents and to avoid such effects, the following actions must be taken: i) identification of neurotoxic potential (hazard) of particular agents, and ii) assessment and management of the obvious risk.

Originally, the overt effects of rather massive doses of today's notorious neurotoxins were a matter of concern. These agents produced severe neurotoxicity resulting in crippling, unbearable pain, blindness, deafness, or dementia. Since such agents have been recognized and appropriate measures for avoiding their effects implemented, interest is focused on subtle effects of chronic, low-level exposure, including potential carcinogenic effects and effects on developing and young organisms, especially the effects on neural functions essential for the human brain, such as learning and memory. The development of sensitive, quantitative and specific tests of cognitive function such as learning and memory has been identified as the highest research priority in neurotoxicology (1). Owing to the quite ubiquitous presence of the nervous system in the organism and to its integrative and steering function with regard to other organ systems, neurotoxicology is deeply involved in other areas of interest, such as endocrine disruption and immunotoxicity.

Extrapolation to humans from experimental animal safety studies

Unfortunately, in the past neurotoxicity was mostly detected in cases of human disaster. Effects of neurotoxins such as methylmercury, organotins, triorthocresyl phosphate, gamma diketone solvents, or hexachiorophene were all initially manifest in humans and only subsequently confirmed in corresponding animal models. In response to this unsatisfactory situation, refined animal models for testing of novel chemicals were elaborated and implemented. The rat was selected as the standard species for testing neurotoxicity, because it is relatively easy to breed, keep and work with, and there is a vast database of pharmaceutical research on rats. However, it has not been demonstrated that the rat exhibits a generally high sensitivity to neurotoxicity. Actually, and unfortunately for dogs, the experience accumulated in our department indicates that the dog may be generally more susceptible to neurotoxicity than the rat.

The animal models presently available comprise highly advanced functional, biochemical and pathological methodologies. For example, the hen model for testing organophosphorus agents for their propensity to induce "delayed neuropathy" (OPIDN) has been developed to utmost perfection by appropriate functional testing, biochemical assay of inhibition of "neuropathy target esterase" (NTE) and examination of the most sensitive brain areas (the termination of spinocerebellar tract) using neuropathology (2, 3). In this model, the degree of NTE inhibition is a quantitative indicator of the biochemical effect of a tested agent, whereas the pathology evaluation reliably shows whether a neuropathy really has occurred. Studies conducted using this methodology show that neuropathic effects characteristic of OPIDN of both type I and type II can be detected with sufficient probability (4). Recent progress in pathological methods and molecular biology provides virtually unlimited technical possibilities. The difficulty lies in the appropriate choice of relevant and feasible methods of investigation. For instance, in the field of developmental neurotoxicity, it has been proposed that the brains of offspring should be submitted to morphometric examination in order to assess the size and cellular composition of particular areas of the brain. Such demands appear exaggerated since this kind of morphometric examination is tedious, and when used routinely would represent a dull exercise of duty. Other approaches, such as functional tests, biochemistry and semiquantitative pathology may be used as primary indicators of possible effects, which, if present, can be additionally characterized by morphometry.

Animal models, needless to say, have limitations due to the morphological, chemical, metabolic and functional differences among species. With respect to the extrapolation to man, there are purely human functions, such as language, which are untestable in animals. What is more, certain reactions of animal organisms have doubtful significance for the extrapolation to humans. An example lies in the alleged neurocarcinogenicity of the artificial sweetener aspartame. Olney *et ald* (5) analyzed National Cancer Institute data

on human central nervous system tumors from 1975 to 1992 and found that there was an increase in brain tumor incidence, which occurred in two phases, the first of which was attributable to the improved diagnostics, but the second more recent one, characterized by a shift toward greater malignancy, was related to an unknown factor. It was suggested that aspartame was a "promising candidate" compared to other environmental factors. The evidence potentially implicating aspartame includes an increased incidence of malignant astrocytic tumors in treated rats. However, one must be aware that there is no unanimity among the experimental pathologists as to the identity of rat astrocytic tumors. The genotype of rat lesions is unknown and their phenotype is hardly comparable to what is understood as astrocytoma in humans (6). Moreover, the observed incidence is dependent on the method of brain collecting and trimming for the examination: laboratories which carefully collect the rostral brain areas, including the olfactory bulbs, and examine multiple brain sections observe more such lesions than those that perform only a cursory examination. Solleveld and Zurcher (7) compared the incidence of astrocytomas in groups of untreated control rats from five different carcinogenicity studies and demonstrated a variation between 0% and 5.9%. There is an obvious need for better tools to assess the carcinogenic potential of chemicals than the expensive and time-consuming rodent experiments that yield equivocal results. As long as rodent carcinogenicity studies are mandatory, it appears desirable to perform them in a way that allows meaningful evaluation, using five or six dose groups in addition to two different control groups. With six dose groups exhibiting a dose-related trend of tumor incidence higher than both control groups, less equivocal evidence of positive carcinogenic effect could be obtained, but the problem of extrapolation among the species would not be solved. Incidentally, the example of aspartame shows that widely spread environmental agents will always be the focus of suspicious attention – recently a warning was distributed on the Internet about aspartame toxicity allegedly causing diseases mimicking multiple sclerosis and systemic lupus.

Interpretation of experimental results
The border between physiology and pathology

It is less difficult to obtain experimental data than to evaluate the data in terms of desirable or undesirable effects. Stimulation of neural receptors can result in their down-regulation, as well as possible changes of postreceptor messenger systems, and development of "pharmacological" tolerance. The tolerant individuals are then less susceptible to the effects of agents stimulating the receptors. Such a situation can occur, for example, with cholinesterase inhibitors. The dilemma for a pathologist then is to decide whether this kind of physiological adaptation can be classified as a pathological condition. Cholinesterase inhibition can improve memory but change sleep pattern (4). When exposed to cholinesterase inhibitors, individuals with deteriorated memory will welcome the improvement, while those with no memory problems may complain about the changes in sleep pattern. Organophosphorus pesticides, especially insecticides, are an example of widely spread environmental cholinesterase inhibitors. They are agents with potent biological activity. Their acute neurotoxic effects are well known, can be treated with medication and the risk of their occurrence is manageable. Recently, these agents were alleged to induce so-called "chronic syndrome" resulting from long-term, low-level, apparently asymptomatic exposure. The clinical features of this alleged syndrome are vaguely defined and include complaints about reduced attention, memory disturbance, reduced velocity of thinking, reduced velocity of psychomotor reactions, impaired dexterity, language disturbance, anxiety, irritability, depression, sleep disturbances, general fatigue and altered sexual habits. Taken together, these complaints very much resemble the changes commonly known to occur with advanced age. They are difficult to recognize as a nosological entity in the individuals who had been exposed for decades to low levels of cholinesterase inhibitors and aged naturally during this period. The controversial issue of chronic syndrome can hardly be solved by pathology, since no pathognomonic lesions were identified. The biochemical data indicate that changes related to cholinesterase inhibition are reversible and therefore physiological rather than pathological.

The impact of recent legislation on the regulation of enyironmental agents

The simultaneous presence of multiple environmental agents gives rise to public concern about the potential toxicity of the combination of agents. An example can be found in recent United States legislation. The Food Quality Protection Act (FQPA), a new regulation setting stringent standards for pesticide residues in foods, became law on August 3,1996. The US Tolerance Reassessment Advisory Committee (TRAC) has identified nine science policy issues associated with its implementation. Among these, two items are of particular interest: aggregating exposure from all nonoccupational sources (exposure to the same agent from all such sources are put together to estimate total exposure), and cumulative risk assessment, especially for organophosphorus pesticides (exposures to various chemicals with common mechanism of toxicity put together). With respect to these items, it is important to consider differences among the chemicals that are apparently identical or appear to have a common mechanism of toxicity.

The following examples demonstrate the pitfalls in classifying agents and their mechanisms of action as "identical".

Mercury

Neurotoxicity of mercury is widely known as Minimata disease, although the original denomination was Hunter-Russel syndrome. This condition is characterized by damage of neurons in the cerebral and cerebellar cortex and the dorsal root ganglia. It is induced by an organic form of mercury, methylmercury. Although inorganic mercury can be deposited in the nervous system, especially after exposure to mercury vapor, and induce neurologic signs, it does not produce any specific pathological lesions (8). It is believed that in order to produce Minimata-like lesions, inorganic mercury would have to be converted into its organic methylated form.

Organotins

There are two neurotoxic trialkyltin agents: trimethyltin produces damage to the cerebral neurons, while triethyltin produces totally different lesions in the edema of white brain matter. Aldridge *etal.* used molecules containing either two methyl and one ethyl group (dimethylethyltin) or vice versa (methyldiethyltin) and demonstrated that such agents produce both kinds of neurotoxicity, whereby the predominating lesion is associated with two identical alkyl groups. However, it has not been demonstrated that addition of trimethyltin could modify the effects of triethyltin, or vice versa.

Hexacarbon solvents

Certain agents with a aliphatic hexacarbon structure, which are mainly used as solvents, are neuropathic. Originally, this neuropathy was denominated "hexacarbon neuropathy". It was then demonstrated that only gamma diketones are neurotoxic, while diketones with other than gamma spacing are not. Therefore, it appears incorrect to consider all hexacarbons to be identical. Moreover, carbon disultide, chemically a quite different molecule, produces the same type ot neuropathy as gamma diketones (10).

Suppression and promotion of OPIDN

Selected chemicals can protect against OPIDN when administered before the neuropathic agents. In contrast, when administered after the neuropathic agents these chemicals aggravate OPIDN (4). The protective effect may be associated with the interaction on the same target (NTE), but the promoting effects probably result from the interaction of promoters with a molecular target other than NTE. This example shows that different mechanisms can be involved in the pathogenesis of one type of lesion, and the order of events determines the level of toxicity, which cannot be assessed by simple cumulating exposure levels.

New horizons in neurotoxicology

The requirement for aggregating and cumulating neurotoxic risks opens interesting, yet unanswered questions. Will simultaneous exposure to inorganic and organic mercury, to ethyl and methyl tin, to gamma diketones and other hexacarbons, or to multiple organophosphorus agents with other agents enhance, reduce, or otherwise modify the neurotoxicity? These questions of combination neurotoxicity cannot be solved without adequate experimental work. Experience shows that neurotoxins can be classitied by their cellular targets (neuronal damage – neuronopathy, axonal damage – axonopathy, myelin damage – myelinopathy, glia cell damage – gliopathy) rather than by their chemical structure and that similar lesions can be produced by chemically different agents. The type and pattern of the lesion is also determined by the type of exposure, as different lesions can be produced by identical agents tollowing short-term exposure to high levels or long-term exposure to low levels (11). The elucidation ot mechanisms instrumental in the production ot toxic neuropathies is the essential task of future activities. Their better understanding will improve the public attitude towards environmental agents, and allow full advantage to be taken of their benefits while avoiding undesired effects.

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Test strategies for the identification of endocrine active chemicals. An industry point of view

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In recent years, possible adverse effects of endocrine active chemicals (EAC) on the environment and human health have been widely discussed in the public and in the scientific community. There is a general consensus that more scientific knowledge is necessary and that EACs should be identified for a refined risk assessment. In this regard, the following three-step approach seems to be the most appropriate:

i) Priority setting or initial assessment to identify from the large universe of chemicals those to be subjected to initial screening.
ii) Screening using a battery of *in vitro* and/or *in vivo* tests to identify chemicals for further detailed testing.
ii) Testing in definitive animal models for risk assessment, including not only adult animals but also *in utero* exposure.

Such a strategy has been proposed to the United States Environmental Protection Agency (EPA) by the Endocrine Disrupter Screening and Testing Advisory Committee (EDSTAC), a multistakeholder advisory group, with participants from authorities, universities, environmental organizations and industry, and has recently been published by the US EPA as its Endocrine Disrupter Screening Program for public comment (EPA, 1998; Fed. Rag., 1998). A similar principle was developed by the Organization for Economic Cooperation and Development (OECD) Working Group on Endocrine Disrupter Testing and Assessment (EDTA).

The screening and definitive test procedures should address possible effects on wildlife and human health. The following will focus on the latter, human health. For the different levels, the tests found below have been proposed or are under discussion.

For priority setting, the US EPA proposes high throughput systems with transfected reporter gene cell lines for estrogens, androgens and thyroid hormones.

For *in vitro* screening, receptor-binding/transcriptional activation assays have been proposed both by the US EPA and the OECD for the *in vitro* part. In addition, the EPA suggests *in vitro* investigations on steroid metabolism.