

Brain dysfunction in animals — Measurements and methods

Rapporteurs: KG Haglid, Per Kjellstrand

Acute administration of 1,1,1-trichloroethane has been shown to increase the levels of adenosine 3',5'-cyclic monophosphate (cAMP) time and dose dependently in the brain stem of mice, while other brain areas were unaffected. Regional differences in the action of 1,1,1-trichloroethane on increased calcium ion influx into synaptosomes were observed and correlated to changes in the cAMP levels (Dr K Nilsson, National Board of Occupational Safety and Health, Solna, Sweden). Furthermore, high doses of 1,1,1-trichloroethane or trichloroethylene increased cerebral blood flow in rats and could be ameliorated by indomethacin, but not by diclofenac, a finding indicating that mechanisms other than prostaglandins were involved. 1,1,1-Trichloroethane, trichloroethylene, and toluene in subanesthetic doses also increased levels of lactate and pyruvate in rat brain, possibly by inhibition at the site of pyruvate dehydrogenase. Such effects could be mediated by changes in the lipid structure of mitochondrial membranes (Dr T Wieloch, University of Lund, Lund, Sweden).

Toluene also increased catecholamine levels and turnover in several hypothalamic nuclei, as well as prolactin secretion. Selective effects on cortical catecholamine neurons were induced by toluene, eg, an increased number of β receptors with a reduced affinity for the transmitter. However, the complexity of interactions between different organic solvents and brain functions was pointed out, since benzene induced a different pattern of changes in distinct hypothalamic nerve cell populations. Following neonatal exposure to toluene, long-lasting reductions of amine turnover were evident in the adult animals (Dr K Fuxe, Karolinska Institute, Stockholm, Sweden).

The inhalation of trichloroethylene at moderate levels (320 ppm) results in biphasic changes in levels of S-100, an astroglial protein possibly involved in the regulation of calcium and zinc ions, phosphorylation, and microtubular disassembly, in several different rat brain areas during eight weeks. The alterations could be explained by an overpowering astroglial cell reactivity comprising increased synthesis and decreased destruction of the protein (Drs KG Haglid and L Rosengren, University of Göteborg, Göteborg, Sweden). The astroglial cells are structurally connected with capillary walls and might create a blood-brain barrier towards the nerve cells and is also a main link in the functional complex of the central nervous system transport of substances. This cell type seems prone to react upon mechanical lesions, but also after the interaction of organic solvents. However, astroglial cells in different brain areas did not respond in a similarly time-dependent way during trichloroethylene exposure. Upon prolonged exposure, even at lower levels of

trichloroethylene, S-100 was decreased as a sign of either inhibited synthesis and/or increased proteolysis.

In identical experiments it was observed that lipid changes occur in the brain after the onset of trichloroethylene inhalation between days 5 and 30. Although major lipid classes did not change at that stage (cholesterol, phospholipid, cerebroside), trichloroethylene induced alterations among highly unsaturated fatty acids in ethanolamine glycerophospholipid (EPG), one of the major phospholipids of the inner surface of cell membranes. A shift was observed towards less unsaturated fatty acids in ethanolamine glycerophospholipid (Dr T Kyrklund, University of Göteborg, Göteborg, Sweden). A possible explanation is a biological adaptation of brain membranes to withstand the fluidizing effect of trichloroethylene. The observed lipid changes varied between different brain areas and seemed to be much more pronounced in brain areas rich in gray matter. Although it has been shown that lipophilic molecules, when chronically incorporated into excitable membranes, induce tolerance so that isomolar doses no longer cause equivalent effects, the time course and plausible mechanism behind this phenomenon is poorly understood.

During subchronic exposure prolonged over weeks, several measurements might show inhibitions caused by some solvents. Ethanol ingestion or benzene exposure leads to decreased S-100 levels after 8–12 weeks. Noticeable is that exposure to benzene mainly in a dose-dependent manner gives alterations in cerebellar areas (Drs Haglid and Rosengren). Benzene intoxication causes a disturbance in the coordination of movements and muscular exercise connected with the nerve cell activity of, eg, the cerebellar region. An important point is thus that different discrete brain areas might not be equally sensitive to different organic solvents, a possibility which could obscure the results of any analysis performed on the whole brain.

Due to the differential sensitivity of different cell compartments of the brain, eg, neuronal versus glial, measurements specific for each cell type are of importance to map neurotoxic effects of organic solvents. The complexity of changes necessitates careful studies with regard to different brain areas, dose, and time over extended investigation periods to explore whether or not a measurement corresponds to the depressant effect induced by organic solvents in the central nervous system. Since nervous system toxicity, induced by industrial chemicals, cannot be explained by a single mechanism, studies of acute and subchronic effects are of great importance to get a more complete classification of the mechanisms involved.

Chronic exposure to organic solvents has been shown to result in irreversible changes which are based on a structural breakdown or cellular alterations in the

central nervous system. Low-dose exposure to different organic solvents (trichloroethylene perchloroethylene, and methylene chloride, 60–350 ppm), as well as high-dose exposure to ethanol (10 g/kg body weight daily) for three months results in astroglial reactions in the form of astroglial hypertrophy and/or proliferation determined four months after the end of exposure (Drs Haglid and Rosengren). Such irreversible changes could be interpreted as long-lasting astroglial reactions due to progressive noxae. Chronic ethanol intoxication decreased the deoxyribonucleic acid (DNA) content of the frontal cerebral cortex. Such a decrease in DNA suggests hypoplastic changes, ie, loss of sensitive cells. Some solvents induced an irreversible loss of DNA in various brain areas without any alterations in glial parameters, this result indicates that sensitive cells had been degenerating without the corresponding glial reactivity exemplified by low-dose exposure to 1,1,1-trichloroethane (Drs Haglid and Rosengren).

The complexity of the irreversible changes due to exposure to different organic solvents seems to reflect the sums of modifications induced by the chemical in different cellular components of the central nervous system. Each investigated solvent had its own pattern with regard to irreversible effects in different brain areas. Aromatic solvents (toluene, benzene) seem to have the cerebellum as the primary target in the low-dose range, but chlorinated aliphatic hydrocarbons, as well as ethanol, also affect the cerebral cortex with increasing sensitivity caudorostrally. Behavioral, electrophysiological, and morphological methods have been used to show that irreversible hearing loss is induced in rats by toluene, xylene, and styrene (Dr G Pryor, SRI International, Menlo Park, United States). Chronic exposure to chlorinated aliphatic hydrocarbons (trichloroethylene and perchloroethylene) altered amino acid levels in distinct brain areas in a specific pattern for each solvent (Dr C Briving, University of Göteborg, Göteborg, Sweden). Irreversible alterations upon exposure to 2,5-hexanediol, eg, increased amounts of neurofilaments, were shown by the aid of monoclonal antibodies (Dr B Bäckström, National Board of Occupational Safety and Health, Solna, Sweden).

The close biotrophic interconnections between different cell types of the central nervous system are still not understood, especially not when chemical agents interfere with normal function. If a chemical agent induces changes in any one of the cell elements of the central nervous system, eg, neurons, oligodendrocytes, astrocytes, or microglia, changes could occur in any other elements of the tissue in question. In this situation almost invariably primary and secondary changes would be shown superimposed on one another. One question is then to identify the cell elements which show the primary lesion initiating the pathogenetic process and to determine the kind of disorder the lesion has sustained.

In the afternoon session Dr P Kjellstrand (University of Lund, Lund, Sweden) made some introductory remarks on the use of animal behavior as a probe for brain dysfunction. It was emphasized that, although it is true that brain damage may lead to alterations in behavior, it does not follow that alterations in behavior must have their primary origin in the brain.

Dr Kjellstrand presented a method for the study of animal motility. The method is based on Doppler radar units coupled to a small desktop computer. The results from studies of acute effects of several different solvents were discussed.

Dr M Junnila (Institute of Occupational Health, Helsinki, Finland) presented studies of low-level exposure on open field behavior. The sensitivity of the classic method was increased by the use of computers. Especially in animals not used to the test situation, effects were seen at low levels of exposure.

The avoidance of signaled bar-press shock had been used by Dr R Kishi (Sapporo Medical College, Sapporo, Japan) to study the effects of toluene and trichloroethylene. Toluene caused a decreased excitation in performance at approximately one-fourth of the brain concentration which induced anesthetic performance decrements.

Dr P Nylén (National Board of Occupational Safety and Health, Solna, Sweden) presented an ongoing two-year study of the effects of three different concentrations of toluene. Several interesting neurophysiological methods are concurrently being used together with studies of behavior.

Dr R Tham (University Hospital, Linköping, Sweden) discussed the vestibulo- and oculomotor response method applied to rats. The method is interesting in that it measures the response from a known brain pathway. There seems also to be a possibility for a closer comparison to the effects on humans than with most other behavioral tests. With this method substances may be grouped according to their effect. The grouping seems to be coupled to the molecular structure of the substance.

An interesting extension and development of the old rotarod method was presented by Dr O Wolthuis (Medical Biological Laboratory, TNO, Rijswijk, The Netherlands). The method, based on a computerized television system analyzing body movements of rats inside a rotating drum, is sensitive and rapid. It seems suitable for repeated measurements and may therefore be a good candidate for screening for toxic effects of solvents.

The workshop was ended by Dr O Ladefoged (National Institute of Occupational Health, Hellerup, Denmark), who discussed the interaction of acetone and 2,5-hexanedione. Several different parameters, such as peripheral nerve conductivity, rotarod performance, and body weight, were used to monitor the effects. The results indicated a neurotoxic potentiating effect between acetone and 2,5-hexanedione. A lively discussion followed on the subject of potentiation.

Liver damage and laboratory diagnostics

Rapporteur: Christer Tagesson

The workshop focused on two major issues: (i) results of cross-sectional and longitudinal studies on liver damage in solvent-exposed workers and the design of future epidemiologic studies and (ii) the evaluation of the individual worker in daily clinical practice and the possible use of various laboratory tests for screening purposes.

Dr I Lundberg (National Board of Occupational Safety and Health, Solna, Sweden) reported on the serum activities of the liver enzymes in 47 paint industry workers and unexposed age-matched referents. The workers were exposed to a mixture of industrial solvents, of which xylene was the main component in most cases. The median total exposure was about 50 % of the Swedish exposure limit values, according to individual solvent exposure measurements performed at the same time. No differences in enzyme activities were revealed, either when the whole exposed and reference groups were compared, or when the five workers with outstanding solvent exposures of five times the exposure limit values or more were compared to their referents. He concluded that the liver seems to remain largely undamaged from inhalation exposure to commonly used mixtures of nonchlorinated solvents and that in many workers this is the case also for very high exposure levels during limited periods.

Dr E Lindberg (National Board of Occupational Safety and Health, Solna, Sweden) referred to a study on perchloroethylene, alcohol, and the influence on liver enzymes. The study included 36 dry cleaners who could be distributed into groups with very different exposure to perchloroethylene and very different alcohol consumption. Workers with "high" exposure to perchloroethylene (mean = 40 ppm) and regular alcohol consumption (mean = 65 g pure alcohol per week) had higher serum activities of the liver enzymes ornithine carbamyl transferase, aspartate aminotransferase and alanine aminotransferase than workers with corresponding alcohol consumption but with "low" exposure to perchloroethylene (mean = 10 ppm). Their enzyme activities were also higher than in workers with "high" exposure to perchloroethylene but low alcohol consumption (mean = 3 g pure alcohol per week). A reduced exposure was followed by decreased enzyme values in serum only in those workers who stated regular alcohol consumption. He suggested that perchloroethylene might potentiate the liver toxicity of alcohol in man, due to its inducing capacity of enzyme systems, as seen in animal experiments.

Dr M Dössing (Gentofte University Hospital, Hellerup, Denmark) discussed liver enzymes and microsomal liver function in polystyrene workers, printing trade workers, and workers exposed to gasoline. As an index of microsomal enzyme activity, antipyrine clearance was estimated by the one-sample saliva method. Antipyrine clearance was inhibited by about

15 % in spray painters during exposure as compared to values obtained at the end of an exposure-free period of three weeks. No difference in antipyrine clearance was found in styrene- and toluene-exposed subjects. He concluded that a more general application of the antipyrine test must await the results of further studies but suggested that it seems prudent to maintain an open mind about the possibility that solvents may be etiologically involved both with regard to various types of liver damage and other adverse health effects.

Professor O Axelson (University Hospital, Linköping, Sweden) agreed with previous speakers in that what is needed is clear-cut, longitudinal epidemiology focusing on the etiologic factors of liver steatosis and cirrhosis and, especially, considering the possibility that occupational exposures, with or without adjuvant alcohol consumption, may play a greater role than recognized as yet. Such epidemiology is difficult, however, and the only reasonable approach seems to be through case-referent (case-control) studies. The proper design of such studies would be more than usually complicated and critical because, in addition to the problem of taking care of alcohol as a confounding factor, several types of occupational exposures might contribute and tend to occur mutually exclusively in the population. In addition it is not sufficient to consider the exposure frequency for just one or the other of the environmental exposures among cases and referents and neglect the rest, since the unexposed category might then contain cases due to the other pertinent exposures. The solution would be a study design distinguishing several (mutually exclusive) categories of potentially hazardous exposures so as to obtain a reference category without effects from more or less foreseeable adverse exposures. Without the application of such a principle in the design, only very widespread risk factors like alcohol can be identified through epidemiologic research, even if various other risk factors operate in small subpopulations and would be rather strong and quantitatively important when taken together. Professor Axelson concluded that it seems natural at this time to take a somewhat critical attitude as to whether or not there is a causal link between exposure to commonly used solvents and liver damage. In addition, it seems unjustified simply to suggest an etiology of alcohol whenever liver damage has been observed without any other well-established cause directly at hand.

The workshop then turned to the second issue. Dr L Ranek (Rigshospitalet, Copenhagen, Denmark) presented a number of cases from the Division of Hepatology at Rigshospitalet. These case histories convincingly showed that occupational exposure to organic solvents may induce histological changes in the liver and give rise to elevated transaminases in serum. He

pointed out, however, that, if no other agents than the solvents are at hand, there is currently little or no evidence to indicate that liver cirrhosis or any other chronic liver disease may develop in due course. He also stressed the point that, as far as we know, the changes seen in the liver are of little or no pathological or pathogenetic significance. On the other hand, the possible effects of combined exposures, eg, organic solvents or other chemicals, alcohol, drugs, etc, is still an open matter and subject to investigation only by large epidemiologic studies.

Professor O Vesterberg (National Board of Occupational Safety and Health, Solna, Sweden) pointed to the possible use of transferrin determination for detecting effects of chemical exposure. Isoelectric focusing in agarose gel has been used for the separation of different molecular forms of transferrin (Tf) in sera and plasma from alcoholics and referents (4). One special form of transferrin with isoelectric point 5.7 (Tf_{5.7}) was quantified with the use of zone immunoelectrophoresis assay, and this method was also used for the quantification of total transferrin (Tf_{tot}). Significant differences in the ratio Tf_{5.7}:Tf_{tot} between the groups were obtained. The results point to the possibility that the determination of a transferrin variant might be a better detection of alcohol abuse than currently used assays. He also suggested that this technique might be of use for detecting early liver damage in subjects occupationally exposed to organic solvents.

Dr C Tagesson (University of Linköping, Linköping, Sweden) discussed the possible use of bile acid determinations in serum for the early detection of hepatic dysfunction and solvent-induced liver damage. Bile acid levels in serum are raised in a variety of hepatobiliary diseases and are apparently a very sensitive indicator of hepatic dysfunction. The question of whether bile acid levels are raised also in the serum of workers exposed to solvents is still unanswered however. Two recent studies concerning the bile acid levels in serum of styrene-exposed workers have given conflicting results. In one of these investigations (2) fasting serum concentrations of conjugated bile acids were investigated in 23 men who had been exposed to styrene; the concentrations were compared with the corresponding concentrations of 60 unexposed individuals. Eleven of the exposed subjects had raised concentrations of either cholic acid or chenodeoxycholic acid or both. There were no indications of alcohol abuse, drug intake, or undiagnosed liver disease in these subjects. These findings point to the possibility that raised bile acid concentrations in serum might be a sensitive and early indicator of hepatic injury in individuals exposed to styrene.

On the other hand, the liver functions, as reflected by serum enzyme activities and by the concentrations of cholic acid and chenodeoxycholic acid, were recently studied in 34 styrene-exposed and 34 reference female workers (3). The results showed that the styrene-

exposed group did not have higher values for any measurements when compared to those of the referents. It appears therefore that the superiority of serum bile acid determinations as an indicator of early liver damage in styrene-exposed individuals is by no means settled. Dr Tagesson concluded that the measurement of bile acid levels in the serum of subjects exposed to organic solvents is likely to remain a research tool, or at least be restricted to centers with a special interest.

Dr C Edling (Karolinska Hospital, Stockholm, Sweden) discussed solvent exposure and liver enzymes in clinical practice and raised the question of whether exposed workers should be examined with regard to liver dysfunction before and during employment. Screening in the general population has shown that the prevalence of pathological liver tests varies between 3 and 30 %, depending on which test has been used. Most of these findings seem to be due to an excess intake of alcohol. Nevertheless, there might be certain risk groups among the exposed who need particular attention. Thus interaction may occur between different solvents and between solvents and alcohol, and it has also been suggested that interaction between drugs and solvents might cause liver dysfunction (1). Moreover, it is possible that long-term exposure increases the risk for raised liver enzymes. One could therefore discuss whether people taking certain drugs or having been exposed for more than 10 years should be regularly monitored.

The working group felt that there was little to support the idea of a general screening for liver damage among solvent-exposed workers, because there is presently little direct evidence in support of the concept that occupational exposure levels in most developed countries are generally harmful to the liver and because available laboratory tests are not sensitive or specific enough. If the result of a routine test for determining liver function is abnormal but the worker has no symptoms of liver disease whatsoever, the laboratory finding is likely to be of little significance and might not be considered. On the other hand, if the results of liver tests are abnormal and the patient shows signs and symptoms of liver disease, the possibility of solvent exposure should be considered, the exposure reduced, and repeated laboratory tests taken after the exposure has been reduced. Finally, the working group felt that if one single laboratory test should be recommended for assessing liver damage due to chemical exposure, the use of a serum transaminase would be the first choice.

The workshop was closed by Dr Dössing, who concluded that, although the problem of occupationally induced liver disease may be of less magnitude than the problems in other organs, the workshop had clearly pointed out the need for further research. Acute and subacute liver injury due to occupational exposure is probably rare, but has been documented and characterized, and the true incidence and prevalence of chronic hepatic injury from workplace exposures are

unknown. Moreover, the methods to identify occupational hepatic injury are still primitive, and possible causal factors in the workplace have been incompletely defined.

References

1. Edling C. Interaction between drugs and solvents as a cause of fatty change in the liver. *Br J Ind Med* 39 (1982) 198—199.
2. Edling C, Tagesson C. Raised serum bile acid concentrations after occupational exposure to styrene: A possible sign of hepatotoxicity. *Br J Ind Med* 41 (1984) 257—259.
3. Härkönen H, Lehtniemi A, Aitio A. Styrene exposure and the liver. *Scand J Work Environ Health* 10 (1984) 59—61.
4. Vesterberg O, Petrén S, Schmidt D. Increased concentrations of a transferrin variant after alcohol abuse. *Clin Chim Acta* 141 (1984) 33—39.

Addresses of the rapporteurs

Carl Gustaf Elinder
Research Department
National Board of Occupational Safety and Health
S-171 84 Solna Sweden

KG Haglid
Institute of Neurobiology
University of Göteborg
PO Box 33031
S-400 33 Göteborg Sweden

Christer Hogstedt
Research Department
National Board of Occupational Safety and Health
S-171 84 Solna Sweden

Gunnar Johanson
Research Department
National Board of Occupational Safety and Health
S-171 84 Solna Sweden

Anders Kjellberg
Research Department
National Board of Occupational Safety and Health
S-171 84 Solna Sweden

Per Kjellstrand
Department of Zoophysiology
University of Lund
Helgonavägen 3 B
S-223 62 Lund Sweden

Per R Nylén
Research Department
National Board of Occupational Safety and Health
S-171 84 Solna Sweden

Christer Tagesson
Department of Occupational Medicine
Linköping University
S-581 83 Linköping Sweden

Jan E Wahlberg
Research Department
National Board of Occupational Safety and Health
S-171 84 Solna Sweden

Olof Vesterberg
Research Department
National Board of Occupational Safety and Health
S-171 84 Solna Sweden