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Neurotoxicity of 1,1,1-trichloroethane questioned

In a study recently published by Karlsson et al (1) young adult Mongolian gerbils (4 males and 4 females) were continuously exposed by inhalation to 1,1,1-trichloroethane at 70 ppm for three months. Under the same experimental design animals were exposed to tetrachloroethylene (60 ppm) and methylene chloride (210 ppm). After a four-month solvent-free recovery period the deoxyribonucleic acid (DNA) concentrations were determined in nine different areas of the brain. In the animals exposed to 1,1,1-trichloroethane, the DNA content was reported to be significantly lower than in concurrent controls in three out of nine areas of the brain, ie, the posterior cerebellar hemispheres, the anterior cerebellar vermis, and the hippocampus. The authors interpreted this finding as an effect of 1,1,1-trichloroethane causing decreased cell density, which may be due to loss of cells. On this basis they concluded that 1,1,1-trichloroethane causes signs of neurotoxicity in different parts of the brain.

The conclusions of Karlsson et al are based on a small number of observations, ie, those on eight controls and eight treated animals. Separating brain lobes with a precision that will prevent cross-contamination (which can have a great influence on the quantitative measurements of DNA) of brain tissue is generally regarded as a difficult task. In view of the small quan-

titative differences in the various brain areas measured by Karlsson et al, it is necessary to have background values to judge whether the values obtained after the 1,1,1-trichloroethane exposure deviate from a solid background data base. As this aspect was not properly discussed by Karlsson et al, we have taken the values from the three parallel control groups as represented in figures 1, 2, and 3 in their paper. By comparing the three control values for each of the brain areas supposedly affected by 1,1,1-trichloroethane with the value obtained after exposure, we found that the alleged DNA reduction in the anterior cerebellar vermis and the hippocampus were just variations within the normal population. The DNA concentration in the posterior cerebellar hemisphere was reported to be significantly lower than the corresponding control value, but it probably does not differ significantly from that of the control group of perchloroethylene, or from the group composed by all the controls and the methylene chloride and perchloroethylene test groups. (See our figure 1). Moreover, the decrease in brain cell density (which should be indicative of cell damage) was not measured in the Karlsson study. The best methodology for this is quantification in histopathological slides, which would identify cell death or necrosis. In the study of Karlsson et al, cell damage was therefore not

Figure 1. Photographic reproduction of parts of figures 1, 2, and 3 of Karlsson et al (1). Deoxyribonucleic acid (DNA) concentration per wet weight (µg/mg) in gerbil brain areas after continuous exposure to 1,1,1-trichloroethane, 70 ppm (T), methylene chloride, 210 ppm (M), or perchloroethylene, 60 ppm (P) for three months followed by a postexposure solvent-free period of four months. [HP = posterior cerebellar hemispheres, VA = anterior cerebellar vermis, HIP = hippocampus. Black columns are average values (and the standard errors of the means) for control animals, striped columns are similar values for the exposed animals.

observed but only claimed via indirect measurement of the DNA concentration in homogenized brain tissues. In addition, the authors' conclusion that exposure to 70 ppm induced a lasting decrease in the DNA concentrations in three brain areas is difficult to understand in the absence of an initial measurement at the end of the exposure period. What happened to the animals in the four-month exposure-free period was not recorded in the paper.

On the basis of the preceding arguments, we do not share the conclusions of Karlsson et al. In our opinion, the claimed effects of 1,1,1-trichloroethane are only variations in a normal distribution, and they do not allow any conclusions with respect to the neurotoxicity of 1,1,1-trichloroethane. The results of a chronic inhalation toxicity and oncogenicity study with rats and mice (exposed for two years to 1500 ppm of 1,1,1-trichloroethane), which did not provide any indications of brain cell damage, support our opinion (2).

References

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Authors' reply

Drs Verschuuren & Wilmer are representatives of the chemical industry. As such, they question the results of our previous paper "Effects of Low-Dose Inhalation of Three Chlorinated Aliphatic Organic Solvents on Deoxyribonucleic Acid in Gerbil Brain."

They claim that our results are due to "variations in a normal distribution." To suppose this statement, they have manipulated our data and constructed a figure displaying mean values of the three separate reference groups and the adherent exposure groups for the three brain regions concerned. By referring to the discrepancy between the mean values of the three reference groups, they then reject our results and state that "they do not allow any conclusions with respect to neurotoxicity..."

This reasoning reveals a severe ignorance of basal statistical procedure for small sample tests, as well as a lack of experience in neurobiological research. It is very evident from our paper that each solvent exposure experiment had its own contemporaneous control group and that the animals were sex-matched littermates. In addition, the different solvent exposures were not performed at the same time but after each other, and, therefore, the different control groups were run at various times of the year. Furthermore, the deoxyribonucleic acid (DNA) analyses were not performed at the same time, the tissues from each solvent exposure experiment being analyzed separately at the end of each experimental period. According to accepted statistical procedure, the results of the analyses were then subjected to evaluation with a test for paired observations, in this case the nonparametric Fisher's permutation test (1). Thus, to compare the different control groups, as done by Drs Verschuuren & Wilmer, is erroneous from a scientific and statistical point of view.

The differences between the mean values of the control groups can easily be explained by the interassay variation of the DNA analyses, the fact that the gerbils used in the different control groups were not matched with respect to each other but with the contemporaneous exposure groups, and the fact that the solvent exposures were performed at different times of the year. However, it is of importance to note that the interassay variation is extremely small and, therefore, allows for statistically significant findings according to a nonparametric test for paired samples (1) even when the quantitative differences observed between the control and exposed animal are small.

Furthermore, Drs Verschuuren & Wilmer consider the number of animals studied small and thus also question the validity of our observations from this point of view. Obviously this point was already considered in the statistical analysis of the data, and the significance levels of the observed changes were set accordingly. However, it should be pointed out that we have previously studied DNA levels after similar exposures but at higher levels for, eg, tetrachloroethylene (2). In this study we observed an even more pronounced decrease in DNA in the hippocampus after exposure at 320 ppm, and a dose-response relation was also found (Spearman's rank correlation: $P < 0.05$) (figure 1). Similar observations have also been made regarding methylene chloride (3).

No studies of DNA concentrations at higher levels of 1,1,1-trichloroethane exposure have been performed. However, in the case of our study, the statistical analysis showed far beyond chance that the DNA concentrations were decreased in three out of the nine studied brain regions after the exposure to 1,1,1-trichloroethane at 70 ppm.

Figure 1. Illustration of results from one of our earlier studies (2). (DNA = deoxyribonucleic acid)

The insinuation of Drs Verschuuren & Wilmer that we do not know how to dissect brain tissue properly is a matter which we feel no need to discuss. It is sufficient to refer to our previous publications in this field (2–6).

The choice of experimental setups and analytical methods to be used in neurotoxicology is of course a matter of dispute. The arguments for our exposure model, using three months of exposure followed by a four-month exposure-free period, have already been discussed in the criticized paper, and also in previous publications (2–8). The obvious advantage of using this exposure model is that changes observed four months after a neurotoxic assault can be considered as long lasting, and possibly irreversible. Regarding the biochemical methods used to assess neurotoxic effects, measurement of DNA concentrations has proved to be a very sensitive indicator of alterations in the nervous system (2–6). We have suggested that the DNA concentration of nervous tissue is an indicator of cell density. Obviously one possible explanation for the observed decreases in DNA after exposure to 1,1,1-trichloroethane at 70 ppm would be the loss of nerve cells.

Drs Verschuuren & Wilmer should also be reminded that previous results from our laboratory have shown very evident effects on the content of a nervous-system specific protein (the glial fibrillary acidic protein) in gerbil brain; these effects indicated astrogliosis of the nervous tissue after similar exposures to 1,1,1-tri-

chloroethane at 210 ppm and 1000 ppm (7). Furthermore, in this experiment (7) the brain weights of the gerbils (which is a very crude but evident indication of neurotoxicity) were significantly reduced ($P < 0.01$) after exposure at 1000 ppm.

We conclude that 1,1,1-trichloroethane is a neurotoxin which induces alterations in brains of experimental animals at exposure levels as low as 70 ppm. Thus, 1,1,1-trichloroethane should not be considered as harmless as representatives of the chemical industry claim it is.

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