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Inhalation exposure to white spirit causes region-dependent alterations in the levels of glial fibrillary acidic protein

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Abstract

Enhanced expression of glial fibrillary acidic protein (GFAP) is known to be associated with toxicant-induced gliosis, a homotypic response of the central nervous system to neural injury. A variety of neurochemical and neurophysiological effects have been observed in experimental animals exposed to white spirit, but a linkage of such effects to neural damage has not been established. Here we evaluated the regional levels of GFAP to assess potential sites of CNS damage in the rat, following exposure to dearomatized and aromatic white spirit. Samples from rats exposed to dearomatized white spirit were assayed for GFAP levels in the United States and Denmark. The results were remarkably similar between countries. Small region-dependent increases and decreases in GFAP were observed with the cerebellum showing the most consistent effects (increases). In contrast, samples from rats exposed to aromatic white spirit showed large (as much as 150% of control) increases in regional levels of GFAP; again, the cerebellum showed the most consistent effects. The data are indicative of an aromatic white-spirit-induced astrogliosis in several regions of the rat CNS and suggest that chronic exposure to this solvent may be associated with underlying neural damage.

Keywords: Gliosis; GFAP; Stoddard solvent

1. Introduction

White spirit (Stoddard solvent, mineral spirits) is widely used as a solvent in paints, printing inks, and varnishes. Various types of white spirit are produced as distillation fractions of crude oil. All are complex mixtures of straight and branched aliphatic, alicyclic, and aromatic hydrocarbons with boiling points in the range of 130–220°C. Various types of white spirit are marketed. The main types are Type 1 (<25% aromatic components), Type 2 (<5% aromatic components), and Type 3, dearomatized white spirit (<1% aromatic components). The complex nomenclature and composition are described in the Environmental Health Criteria Document [10].

The neurotoxicity of white spirit (Stoddard solvent) in humans has recently been reviewed [10]. The nervous system is the critical target organ for organic solvent toxicity. In addition to acute narcotic effects at high exposure levels, chronic effects on the central nervous system have been demonstrated by epidemiological studies in workers exposed to various mixtures of organic solvents [1–3,9,18,19,25]. The effects include impairment of memory and coordination, concentration difficulties, general irritability, and abnormal fatigue. The effects on the central nervous system may persist even when exposure has ceased [7]. Exposure to organic solvents also has been associated with Alzheimer's disease [11]. A recent study on dockyard painters support the hypothesis that heavy and prolonged exposure to paint solvents leads to neuropsychological ill health [6].

In experimental animals, changes in global, regional, and subcellular neurotransmitter metabolism indicate certain acute and long-lasting effects from exposure to aromatic white spirit [15,17,35]. Long-lasting electrophysiological changes demonstrate the neurotoxic potential of dearomatized white spirit [23]. A reduced yield of synaptosomal protein per gram brain weight has been found following exposure to aromatic white spirit for 3 weeks and 6 months [15], which may reflect reduced synapse density and number. Three weeks' exposure of rats to dearomatized white

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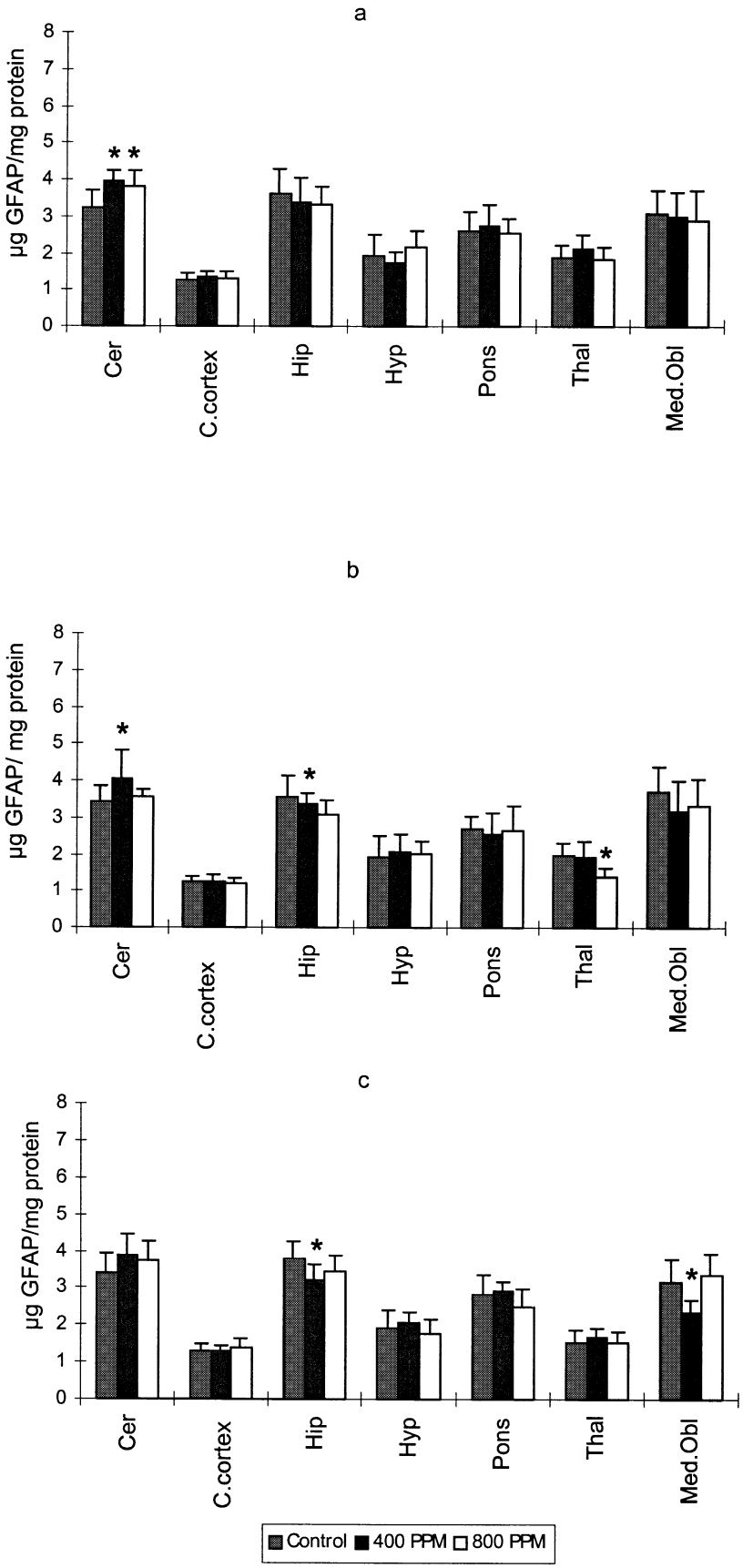
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µg GFAP/mg protein
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c
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Control
400 PPM
800 PPM

Table 1
Body weight (in grams) of rats exposed to 0 (control), 400, or 800 ppm of dearomatized white spirit in the inhaled air for 6 h/day, 7 days/week for 1, 2 or 4 weeks

	Control	400 ppm	800 ppm
1 week	377±23	386±19	388±18
2 weeks	396±39	389±29	386±23
4 weeks	403±19	413±25	408±25

Mean±SD, $n=10$.

spirit induced increased indices of oxidative stress in synaptosomes [16] whereas pro-oxidant events were elevated in kidney and liver, but not in the brain synaptosomes by the aromatic type of white spirit [4].

Previously, we discussed whether aromatic versus dearomatized white spirit had the greatest potential to induce neurotoxicity [12,15,17,23,24]. The health effect of white spirit is hypothesized to depend mainly on the aromatic components, and a substitution with dearomatized white spirit has been proposed. However, there is no scientific data to support the preference of either type concerning long-term effects. During the past years low aromatic petroleum products have been increasingly used based on the presumed lower acute narcotic effects, lesser mucous membrane irritation, lower vapor pressure and lower blood:air partition coefficients of the aliphatic components. In these products the aromatic hydrocarbons are removed or more commonly converted to the corresponding alicyclic hydrocarbons by catalytic hydrogenation.

Damage to diverse regions and cell types of the CNS engenders an astroglial reaction at the site of injury, as assessed by qualitative or quantitative analysis of the astrocyte intermediate filament protein, glial fibrillary acidic protein (GFAP). Using an immunoassay for GFAP, we have demonstrated that enhanced expression of this protein serves as a marker of the dose-, time- and region-dependent damage that results from exposure to broad classes of known and suspected neurotoxic agents [26,28]. Induction of GFAP occurs not only after neurotoxicant-induced cell loss (e.g. after trimethyltin, [5]) but also after more subtle damage, such as loss of a small percentage of nerve terminals in a specific brain region (e.g. after MPTP [29,30]. Despite the fact that large numbers of chemical toxicants have been shown to cause gliosis and an accompanying increase in GFAP, the utility of this protein as a biomarker of neurotoxicity associated with very subtle structural damage to the CNS is less well documented.

The aim of the present study was to investigate the potential neurotoxic effects of dearomatized white spirit by analyzing the GFAP concentration in various brain regions at various times following exposure by inhalation. We also sought to verify the cross-laboratory validity of the GFAP assay by analyzing the same samples in two different laboratories. Additionally, we analyzed regional GFAP levels in brains from rats subjected to inhalation exposure of aromatic white spirit for 3 weeks. The neurotoxic effects of various solvents also have been examined by Rosengren and coworkers using a different method for estimation of levels of GFAP [31–33].

2. Methods

2.1. Chemicals

Dearomatized white spirit (Type 3) was purchased from Exxon Chemical, Denmark (Exxsol D 40, CAS No. 64742-48-9). The boiling interval was 145–200°C, and the aromatic content less than 0.4 wt.%. The mean molecular weight was 143 g/mol. Aromatic white spirit (Type 1), K-30 (CAS No. 64742-88-7) was purchased from Shell, Denmark. The boiling interval was 148–200°C, 20 vol.% aromatics. The mean molecular weight was 140 g/mol.

2.2. Animals

Male rats (Mol:WIST), mean body weight 350 g, 3 months old, obtained from Møllegaard Breeding Center, DK-4623 L1, Skensved, Denmark, were used. The rats were weight-randomized into three groups. They were housed in stainless steel wire cages, two animals per cage, conventionally in animal rooms with automatic control of temperature (22±1°C), relative humidity (55±5%), air exchange (eight times per hour), and fluorescent light (9 p.m.–9 a.m.), with access to commercial pelleted diet (Altromin 1324, Brogården, Gentofte, Denmark) and acidified tap water in nipple bottles. The tap water was acidified with citric acid to pH 3.0. During daily exposure the food was removed. Animals were visually inspected on a daily basis; body weights were recorded before the start of exposure, and immediately before sacrifice.

2.3. Exposure

The exposure schedule for dearomatized white spirit was as follows: One group was sham-exposed (control), the second group was exposed to 400 ppm (2339 mg/m^3), and the third group received 800 ppm (4679 mg/m^3) of dearomatized white spirit in the inhaled air for 6 h/day, 7 days/week for 1 (10 rats/group), 2 (10 rats/group), or 4 weeks (10 rats/group). The exposure schedule for aromatic white spirit was as follows: One group was sham-exposed (con-

Fig. 1. Regional concentration in cerebellum (Cer), cerebral cortex (C. cortex), hippocampus (Hip), hypothalamus (Hyp), pons, thalamus (Thal), and medulla oblongata (Med. Obl.) of GFAP (μg GFAP/mg protein; mean±SD, $n=10$ in all regions except control cerebellum when exposed 2 weeks, $n=9$; determined in the US) for rats exposed to 0 (control), 400, or 800 ppm of dearomatized white spirit in the inhaled air for 6 h/day, 7 days/week for 1, 2, or 4 weeks. (a) 1 week of exposure; (b) 2 weeks of exposure; (c) 4 weeks of exposure.

trol), the second group was exposed to 400 ppm (2290 mg/m^3), and the third group received 800 ppm (4581 mg/m^3) of aromatic white spirit in the inhaled air for 6 h/day, 7 days/week for 3 weeks (10 rats/group). The inhalation exposure conditions and the equipment used have previously been described [13]. Control of the concentration in the inhaled air was performed continuously by use of a Miran 402, Foxboro UK, infrared spectrophotometer.

2.4. GFAP analysis

The day after the end of exposure, the animals were sacrificed by decapitation in CO_2 anesthesia. Brains were dissected into seven brain regions (cerebellum, cerebral cortex, hippocampus, hypothalamus, pons, thalamus, and medulla oblongata) according to the method described by Glowinski and Iversen [8]. Tissue was transferred to ice-cold 0.32 M sucrose, weighed and homogenized. For samples obtained after exposure to dearomatized white spirit, one aliquot of the 1- and 4-week exposure samples was analyzed in Denmark whereas aliquots of all of the samples were also analyzed in the US. All of the samples obtained after exposure to aromatic white spirit were analyzed in Denmark. Both laboratories used the method of O'Callaghan [27] which included the initial heat denaturing step in 1% SDS. In Denmark, purified bovine GFAP

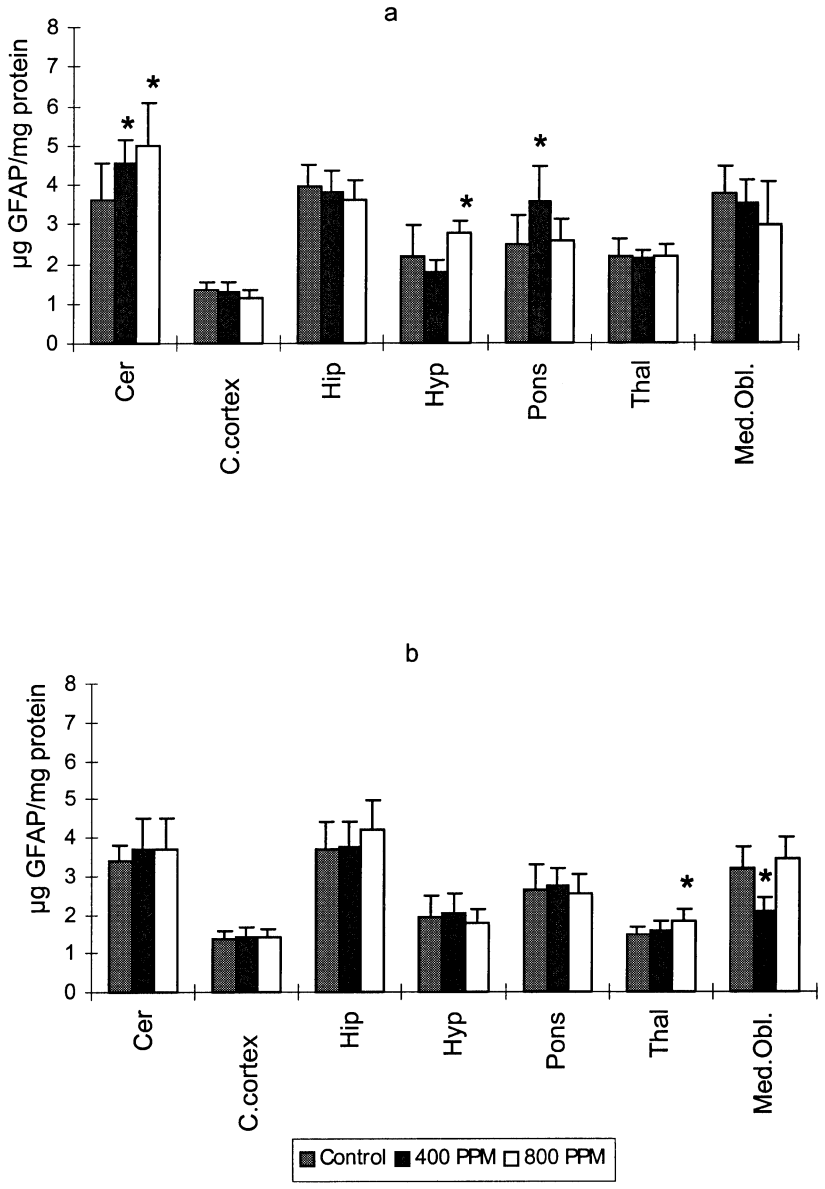

Fig. 2. Regional concentration in cerebellum (Cer), cerebral cortex (C. cortex), hippocampus (Hip), hypothalamus (Hyp), pons, thalamus (Thal), and medulla oblongata (Med. Obl.) of GFAP (µg GFAP/mg protein; mean±SD, $n=10$; determined in Denmark) for rats exposed to 0 (control), 400, or 800 ppm of dearomatized white spirit in the inhaled air for 6 h/day, 7 days/week for 1 or 4 weeks. (a) 1 week of exposure; (b) 4 weeks of exposure.

(Boehringer Cat. no. 981 150) was used for GFAP standard. In the US GFAP values were based on a standard purified from a cytoskeletal preparation of rat spinal cord [29,30].

2.5. Protein analysis

Protein concentration was measured by use of a commercially available kit (Pierce, BCA protein assay reagent No. 23225). In Denmark, bovine serum albumin Sigma A 7030 was used as the standard. In the US, bovine serum albumin fraction V (Sigma A 7906) was used as a standard.

2.6. Statistical analysis

Body weight after exposure, weights of whole brain, and brain parts were analyzed by analysis of variance followed by Dunnett's test where indicated (SAS PC version software package [34]). For analysis of the GFAP data, the Danish samples of week 2 could not be analyzed because of technical analytical problems. Therefore, only data from weeks 1 and 4 could be used for statistical comparison between laboratories (two-factor analysis, SAS).

The general acceptance level of significance was $p<0.05$.

3. Results

3.1. Mortality, morbidity, and subjective behavioral signs

None of the subjects died following exposure to either solvent. During the first week of exposure to either solvent, the rats showed signs of irritation of mucous membranes, and appeared to be sedated. These effects gradually diminished during the second week.

3.2. Body and brain weights

The body weights of rats exposed to 0 (control), 400, or 800 ppm of dearomatized white spirit for 1, 2, and 4 weeks (Table 1) and to the same concentrations of aromatic white spirit (data not shown) were not significantly affected by the treatments. Weights of whole brain and of the brain regions analyzed for GFAP also were not affected by exposure to either agent (data not shown).

3.3. GFAP immunoassay

After exposure to dearomatized white spirit, the most consistent effect observed was a slight increase in the concentration of GFAP in cerebellum. Increases in cerebellar GFAP were seen at 1 and 2 but not 4 weeks of exposure (US data, Fig. 1). When the 1- and 4-week samples were assayed in Denmark (DK), GFAP increases also were observed in the cerebellum at 1 week, but not at the 4-week time point (Fig. 2. DK data). In contrast to the data obtained for cerebellum, dearomatized white spirit caused either decreases (hippocampus, thalamus, medulla oblongata) or increases (pons, hypothalamus, thalamus) (Fig. 1 and Fig. 2, i.e. US vs. DK data). None of the data for these brain regions, whether assayed in Denmark or the US, showed consistent time- or exposure-related relationships. Slightly higher variability of the GFAP assay in Denmark compared to US appear to account for the failure to reach statistical significance at various post-exposure time points and levels of exposure. Overall, however, the results were very similar

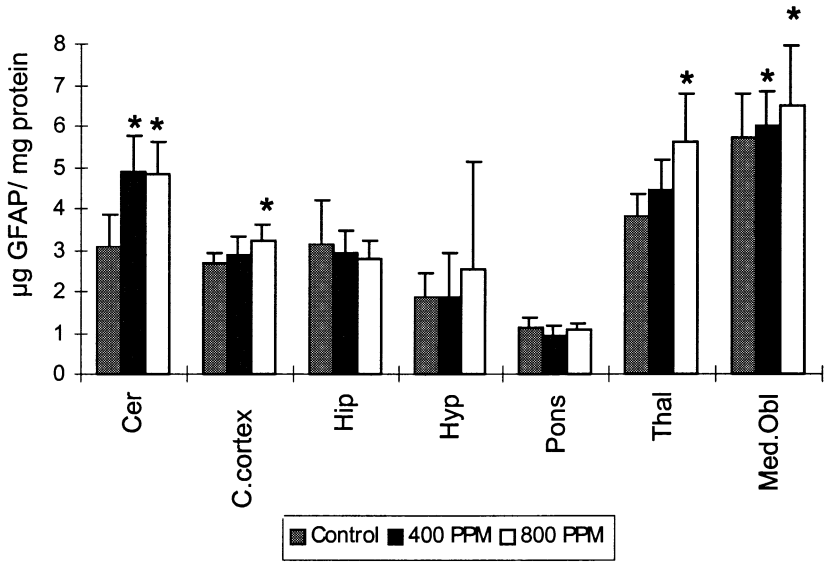

Fig. 3. Regional concentration in cerebellum (Cer), cerebral cortex (C. cortex), hippocampus (Hip), hypothalamus (Hyp), pons, thalamus (Thal), and medulla oblongata (Med. Obl.) of GFAP (µg GFAP/mg protein; mean±SD, $n=10$; determined in the Denmark) of rats exposed to 0 (control), 400, or 800 ppm of aromatic white spirit in the inhaled air for 6 h/day, 7 days/week for 3 weeks.

between Denmark and the US; there were no statistically significant differences (two-factor analysis) between the results obtained in the two laboratories.

As with dearomatized white spirit, aromatic white spirit increased the concentration of GFAP in cerebellum (analysis only performed in DK, Fig. 3) following three weeks of exposure to 400 and 800 ppm. Exposure to 800 ppm increased the GFAP concentration in cerebral cortex, thalamus, and medulla oblongata; whole brain levels were increased at both 400 and 800 ppm.

4. Discussion

Following inhalation exposure to dearomatized white spirit, the most consistent effect on GFAP was a small (20%), time-dependent *increase* in cerebellum. *Decreases or increases* in GFAP of similar magnitude, however, also were observed in thalamus, hypothalamus, hippocampus, pons and medulla oblongata. These were marginal changes (biologically nonsignificant) at best and none of the data follow exposure- and time-dependent relationships that are consistent with our previous data on toxicant-induced increases in GFAP linked to minor structural damage of the CNS [22]. It is tempting to speculate that the cerebellar effects of dearomatized white spirit are indicative of low-level reactive gliosis associated with low-level neural damage in the absence of overt cytopathology as has been observed with other solvent exposure paradigms [31–33]. However, the lack of a clear-cut exposure–response relationship for the GFAP data argues against this possibility. Moreover, although we have seen changes in brain electrophysiology [23], neurochemistry [16], and behaviour [23] after 6 months' exposure to this solvent, none of these effects were specific for cerebellum. Finally, equal weight must be given to the possibility that exposure to dearomatized white spirit causes a true region-dependent decrease in GFAP, findings consistent with at least one other report for solvent exposures [21]. Overall, the small and generally inconsistent effects of dearomatized white spirit on GFAP suggests that the observed changes cannot be definitively linked to reactive gliosis, the hallmark of chemical, trauma, or disease-induced damage of the CNS [20,26]. Thus, previously published neurochemical and behavioral effects of dearomatized white spirit using the exposure paradigm presented here, likely are a reflection of neuroactive effects of this compound in the absence of structural damage to the CNS.

In contrast to the data obtained for dearomatized white spirit, aromatic white spirit resulted in consistent and generally dose-dependent increases in GFAP. As with dearomatized white spirit, the cerebellum appeared to be the most sensitive brain area, although effects as large as 150% of control were seen in other areas (thalamus). Although the data did not reach statistical significance, the same trend was found in a previous study, also with a 3-week exposure period [14]. Six months of exposure to aromatic white spirit followed by an exposure free period of 3 months' duration did not affect the GFAP concentration in any brain region [14]. Given that gliosis and accompanying elevations in GFAP resolve over time after neurotoxic insult [20,26], raises the possibility that this previous study may have missed an earlier and transient rise in GFAP. As with dearomatized white spirit, we have seen changes in brain neurochemistry, whereas behavior was unaffected after the 6-month exposure period [35]. These findings may indicate the reversibility of some measures of exposure to aromatic white spirit and the persistence of others. Unfortunately, data for the two types of white spirit are not easy to compare due to the different sampling time point used in the present study, but the data do indicate that the aromatic type has a greater effect on GFAP expression than the dearomatized type. In view of the fact that elevations in GFAP are suggestive of underlying neural damage, the persistent and previously identified neurochemical changes associated with exposure to aromatic white spirit may reflect long-term changes linked to the initial insult.

Several fairly discrete areas of the CNS, such as hippocampus and hypothalamus, were not affected by exposure to aromatic white spirit. The fact that cerebral cortex and whole-brain levels of GFAP were elevated suggests that specific structures not examined in this study contribute to the elevations in GFAP seen in these latter samples. These observations dictate the need for a more discrete analysis of regional levels of GFAP, perhaps combined with a qualitative examination of this protein by immunohistochemistry, in order to pinpoint all of the target areas potentially damaged by this solvent.

The GFAP assay results for tissue prepared from rats exposed to dearomatized white spirit were remarkably similar between Denmark and the US. The nominal differences observed between the two laboratories easily could be attributed to the use of different GFAP standards which, along with different standards used in the total protein assay, were the only factors that varied between the protocols followed in each laboratory. The strong concordance between the GFAP data obtained in the two countries lends testimony to the utility of the GFAP assay for reliable detection and quantification of potential sites of damage associated toxic exposures of the CNS.

In summary, we have demonstrated that dearomatized and aromatic white spirit affects the regional levels of GFAP. The inconsistent effects of the former compound, verified in two laboratories, do not allow us to conclude that an exposure-related induction of gliosis has occurred. In contrast, the effects of the latter compound on GFAP are suggestive of an exposure-linked induction of astrogliosis. These data are indicative of aromatic white-spirit-induced neural damage in several regions of the CNS, findings that need to be confirmed by complimentary and alternative methods for assessment of neurotoxic responses.

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