Target tissues for two methylsulphonyldichlorobenzene isomers in the upper and mid respiratory tract

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Introduction: Methyl sulphone metabolites ($MeSO_2$-) of PCB and DDT have attracted interest due to their persistency in biota and their ability to be selectively enriched in target cells and tissues in experimental animals [1-3]. Several $MeSO_2$-PCBs are enriched in non-ciliated bronchiolar (Clara) cells following reversible binding to a secretory PCB-binding protein residing in these cells [4,5], but the DDT metabolite 3-methylsulphonyl-DDE is covalently bound in the adrenal zona fasciculata cells following bioactivation by a tissue-specific cytochrome P450 form [6,7]. While $MeSO_2$-DDE is a highly potent toxicant to the adrenal zona fasciculata, the enrichment of $MeSO_2$-PCBs in the Clara cells have not yet been associated with overt toxicity.

In addition a number of polychlorinated benzenes are known to be transformed to methyl sulphone metabolites [8]. In order to determine patterns of distribution of such compounds, a number of ^{14}C-labelled $MeSO_2$-chlorobenzenes have been synthesized [9]. Based on results from an initial autoradiographic screening some compounds have been selected for extended studies with regard to possible site-specific metabolic activation, covalent tissue-binding and toxicity.

We report here that two isomeric compounds, (^{14}C)methylsulphonyl-2,5-dichlorobenzene ((^{14}C)methylsulphonyl-2,5-DCB) and (^{14}C)methylsulphonyl-2,6-dichlorobenzene ((^{14}C)methylsulphonyl-2,6-DCB), give rise to pronounced labelling of the upper and/or mid respiratory tract following administration to mice. In addition, histopathological data showing that the 2,6-chlorinated analog is a potent toxicant to the nasal olfactory mucosa in mice are presented.

Materials and methods: (^{14}C)Methylsulphonyl-2,5-DCB and (^{14}C)methylsulphonyl-2,6-DCB (spec. act. 55 mCi/mmol; radiochemical purity about 98 %) and unlabelled methylsulphonyl-2,6-DCB (98 % pure) were prepared using published procedures [9].

Female C57 black mice (21 g) were given iv injections of the labelled substances (about 0.8 mg/kg; in 20 µl DMSO per mouse) and were then killed at time points ranging from 20 min - 4 days after dosing. Tape section autoradiography was performed according to Ullberg [10]. To determine irreversibly bound radioactivity in the tissues, freeze-dried tape sections were extracted stepwise in 50 % ethanol, 70 % ethanol, 99 % ethanol, heptane and water before being mounted on to X-ray film [6].

Histopathological examination of methylsulphonyl-2,6-DCB-induced lesions was done using methacrylate embedded, decalcified nasal tissue, stained with toluidine blue. Series of female C57 Black and NMRI mice (21 g) were given single ip doses of the sulphone, dissolved in corn oil , and were then killed at time points ranging from 6 hours to 21 days.

Results

Autoradiography : The distribution pattern of (^{14}C)methylsulphonyl-2,5-DCB and (^{14}C)methylsulphonyl-2,6-DCB in mice were fairly similar. During the first four hours after dosing of each compound, a selective localization of radioactivity was observed, particularly in the nasal olfactory mucosa and the lateral nasal glands, but also in the tracheo-bronchial mucosa.This pattern of distribution became more pronounced after 24 hours when in addition a distinct labelling of the olfactory bulb and the kidney cortex was visible for both compounds. Four days after dosing the autoradiograms were characterized by a high and selective retention of radioactivity in the olfactory mucosa, the lateral nasal gland (Figure 1B), the tracheo-bronchial mucosa and the kidney cortex. The 2,5-chlorinated isomer showed a different, more spotty, labelling of the kidney cortex than the 2,6-chlorinated isomer.

Autoradiograms obtained from solvent-extracted tissue-sections revealed that a large part of the (^{14}C)methylsulphonyl-2,6-DCB-derived radioactivity could not be dissolved from the respiratory tract (Figure 1A). Extracted sections from mice dosed with (^{14}C)methylsulphonyl-2,5-DCB were not available.

Histopathology: As demonstrated in a dose-finding study, methylsulphonyl-2,6-DCB induced necrosis in the nasal olfactory mucosa following the lowest single dose so far examined (32 mg/kg). In order to characterize the development and regeneration of the

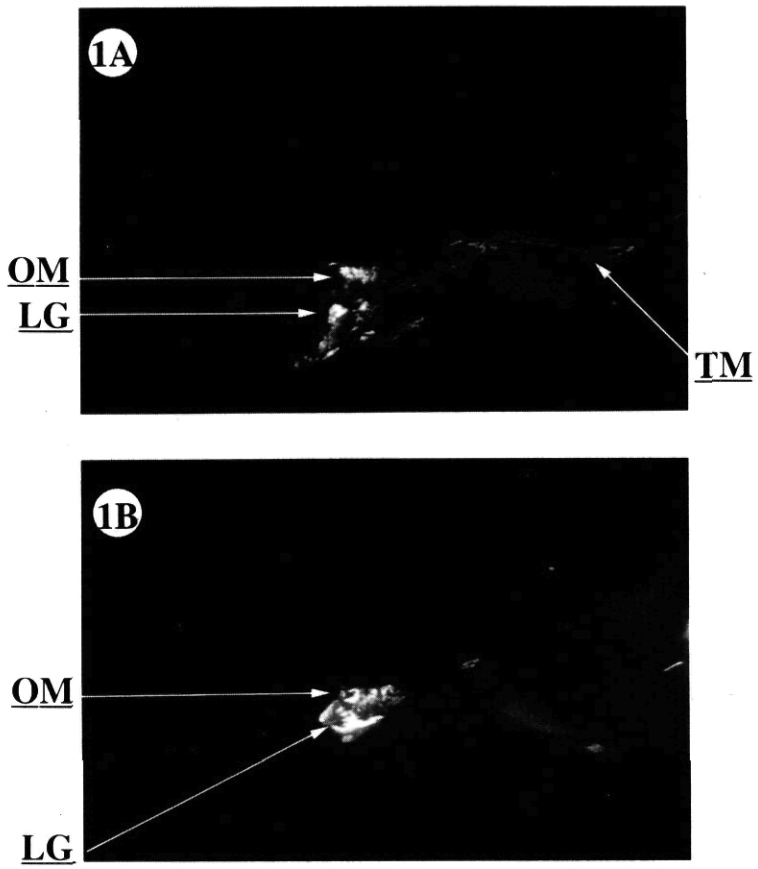

Fig. 1. Autoradiograms of the head regions of mice showing the distribution of radioactivity. 1A represents an extracted tape section of a mouse 24 hr after an iv injection of labelled methylsulphonyl-2,6-DCB. 1B represents a nonextracted tape section of a mouse 4 days after an iv injection of methylsulphonyl-2,5-DCB. Both compounds give rise to a high accumulation of radioactivity in the olfactory mucosa and the lateral nasal glands. (OM, olfactory mucosa; LG, lateral nasal glands; TM, tracheal mucosa).

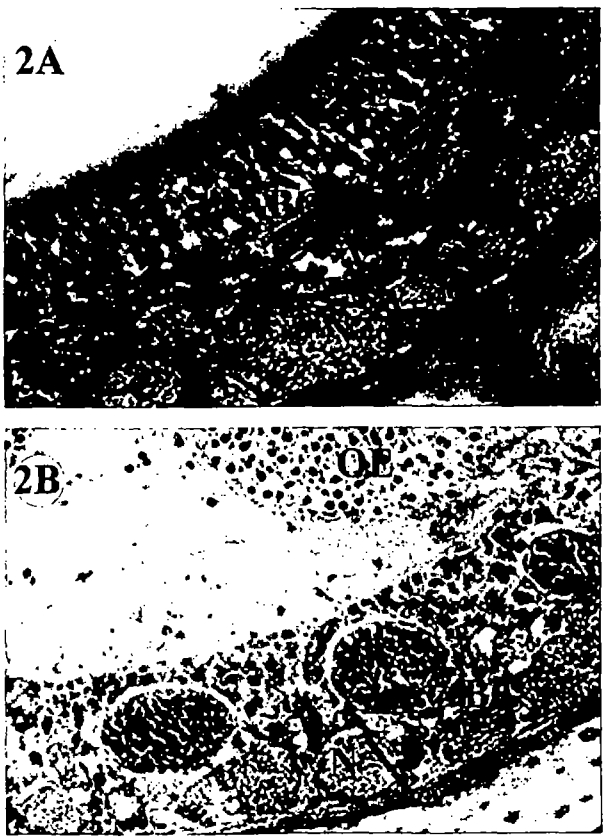

Fig. 2. Histological sections through the olfactory mucosa of mice. 2A represents a control mouse while 2B represents a methylsulphonyl -2,6-DCB (65kg/mg) treated mouse. 2B shows extensive damage in the olfactory mucosa: The Bowman's glands have become completely necrotic; the olfactory neuroepithelium has degenerated and detached from the basal membrane; the blood vessels have dilated; the nerve bundles and blood vessels remain intact. (OE, olfactory epithelium; BG, Bowman's glands; N, nerve bundles; V, blood vessels; toludine blue).

toxic lesion over time, groups of mice were given 65 mg/kg. Six hours later the Bowmans glands were necrotic and degenerative changes were seen in the olfactory neuroepithelium (OE). Twentyfour hours after dosing the neuroepithelium was severely degenerated or necrotic (Figure 2B); three days after dosing the neuroepithelium was completely detached from the lamina propria leaving a nude basal membrane. Seven days after dosing there were signs of fibrosis in the lamina propria, which became even more pronounced after three weeks . Throughout these time points the olfactory neuroepithelium had been replaced by an atypical ciliated epithelium. Hence, there were no evidence of successful regeneration of the olfactory neuroepithelium during the investigative period.

Discussion: The present study shows that methylsulphonyl-2,6-DCB is a potent toxicant that induces necrosis in the olfactory mucosa in mice following exposure to a single dose of 32 mg/kg, the lowest dose examined. The lesion seemed to originate in the subepithelial Bowman's glands which became necrotic within a few hours after dosing. The affected area developed gradually, resulting in degeneration and detachment of the olfactory neuroepithelium within three days. Interestingly, the regeneration of the lesion during the following weeks seemed to result in a replacement of the olfactory neuroepithelium with a ciliated atypical epithelium. Moreover, there were only weak signs of regeneration of the Bowman's glands while the lamina propria showed fibrosis that became very prominent with time. As judged from these histological observations it seems unlikely that a functional olfactory mucosa could be recovered in the methylsulphonyl-2,6-DCB-exposed animals.

The autoradiograms obtained from the two isomeric compounds were strikingly similar with regard to the binding pattern in the respiratory tract. The results obtained from solvent-extracted tissue-sections from mice dosed with (^{14}C)methylsulphonyl-2,6-DCB strongly indicate that the high accumulation and retention observed in the olfactory mucosa was due to an irreversibly bound metabolite, most likely formed in situ. Despite the fact that no extracted sections were available for the 2,5-chlorinated isomer, it seems possible that methylsulphonyl-2,5-DCB may also be present as an irreversibly bound residue in the olfactory mucosa. Methylsulphonyl-2,5-DCB, a lipophilic metabolite formed from the odorant and insecticide 1,4-dichlorobenzene, should thus be considered as potentially toxic to the nasal olfactory mucosa.

The autoradiograms obtained 4 days after dosing with (^{14}C)methylsulphonyl-2,5-DCB were characterized by a selective retention in the bronchial mucosa, the lung tissue and in spotted sites in the kidney cortex. This pattern of distribution is almost identical to that previously observed in mice dosed with the PCB metabolite 4,4'-bis-(methylsulphonyl)-

2,2',5,5'-tetrachlorobiphenyl [4], a compound that binds with high affinity to a secretory PCB-binding protein residing in the non-ciliated bronchiolar (Clara) cells [4,5]. Considering the structural similarities between these methyl sulphones (the PCB metabolite consists of two methylsulphonyl-2,5-DCB molecules connected by a biphenyl bond) it appears possible that the long term distribution pattern of (^{14}C)methylsulphonyl-2,5-DCB represents its association with the PCB-binding protein.

In conclusion, the present study has shown that methylsulphonyl-2,6-DCB is a potent toxicant to the nasal olfactory mucosa in mice, most likely following in situ metabolic activation to a reactive, tissue-binding metabolite. The limited dispositional data available for the 2,5-chlorinated isomer suggest the possibility that methylsulphonyl-2,5-DCB is also a nasal toxicant in mice. The long term binding of methylsulphonyl-2,5-DCB in lung and kidney cortex is indicative of a $MeSO_2$-PCB type of reversible tissue-binding that has not been associated with overt toxicity.

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