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# Derivation of an occupational exposure limit for an inhalation analgesic methoxyflurane (Penthrox®)

John Frangos*, Antti Mikkonen, Christin Down

*Golder Associates, 570 – 588 Swan Street, Richmond, Victoria, 3121, Australia*

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ABSTRACT

Methoxyflurane (MOF) a haloether, is an inhalation analgesic agent for emergency relief of pain by self administration in conscious patients with trauma and associated pain. It is administered under supervision of personnel trained in its use. As a consequence of supervised use, intermittent occupational exposure can occur. An occupational exposure limit has not been established for methoxyflurane. Human clinical and toxicity data have been reviewed and used to derive an occupational exposure limit (referred to as a maximum exposure level, MEL) according to modern principles. The data set for methoxyflurane is complex given its historical use as anaesthetic. Distinguishing clinical investigations of adverse health effects following high and prolonged exposure during anaesthesia to assess relatively low and intermittent exposure during occupational exposure requires an evidence based approach to the toxicity assessment and determination of a critical effect and point of departure. The principal target organs are the kidney and the central nervous system and there have been rare reports of hepatotoxicity, too. Methoxyflurane is not genotoxic based on in vitro bacterial mutation and in vivo micronucleus tests and it is not classifiable (IARC) as a carcinogenic hazard to humans. The critical effect chosen for development of a MEL is kidney toxicity. The point of departure (POD) was derived from the concentration response relationship for kidney toxicity using the benchmark dose method. A MEL of 15 ppm (expressed as an 8 h time weighted average (TWA)) was derived. The derived MEL is at least 50 times higher than the mean observed TWA (0.23 ppm) for ambulance workers and medical staff involved in supervising use of Penthrox. In typical treatment environments (ambulances and treatment rooms) that meet ventilation requirements the derived MEL is at least 10 times higher than the modelled TWA (1.5 ppm or less) and the estimated short term peak concentrations are within the MEL. The odour threshold for MOF of 0.13 –0.19 ppm indicates that the odour is detectable well below the MEL. Given the above considerations the proposed MEL is health protective.



## 1. Introduction

Methoxyflurane (MOF) a haloethane, is clear colourless liquid with a strong sweet fruity odour; CAS no. 76-38-0; MW.164.97 and specific gravity 1.4262 at 20 °C. It has a relatively low vapour pressure (relative to other inhalation anaesthetics) of 3 kPa, a very high blood:gas partition coefficient of 12 and a very high oil:gas partition coefficient of 970 (DOSE, 1994; Goodman and Gilman, 1980).

Methoxyflurane was first introduced to clinical practice as an inhalation anaesthetic in 1960 (Goodman and Gilman, 1980). Its clinical use as an anaesthetic was discouraged in the late 1970's to early 1980's due to reports of dose-related renal tubular damage (Crandell et al., 1966; Mazze et al., 1971, Mazze, 2006) and the availability of newer anaesthetic agents. This led to its reduced use and disappearance from anaesthetic practice by the late 1970s and ultimately its discontinuation in the United States and Canada in the 1990s. In September 2005, in responding to a Citizen's Petition the US Food and Drug Administration determined that Penthrane (Abbott Laboratories' methoxyflurane) was withdrawn from the market for reasons of safety or effectiveness (US FR, 2005). Methoxyflurane was never withdrawn in Australia and New Zealand where it has been available at all times as Penthrox® for use in lower doses as a rapid-acting analgesic for short-term pain relief. Since 1975, over five million MOF doses have been supplied. It is used by ambulance services as a first-line analgesic agent and in

* Corresponding author.
*E-mail address:* jfrangos@golder.com.au (J. Frangos).

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brief surgical procedures such as bone marrow biopsies, colonoscopies and change of wound dressings. It is also supplied in the UK, South Africa, the Gulf Area, Eastern Europe, and Latin America.

Methoxyflurane is self-administered under the supervision of trained personnel, by inhalation from a simple hand held green pen-shaped plastic device, called the 'Penthrox Inhaler' or the 'Green Whistle'. The device is supplied with or without an activated carbon (AC) chamber to reduce environmental exposure. Methoxyflurane may be used in open spaces such as roadsides, sports and ski fields, or in more confined spaces such as ambulances, doctor's surgeries, clinics and emergency departments.

Although some countries list an occupational exposure standard for MOF this is identical to the NIOSH standard (1977) for waste anaesthetic gases. The NIOSH standard, besides its age (published in 1977), is of limited relevance to the analgesic use of MOF. It was set to protect against co-exposure to all inhalation anaesthetics in operating theatres, including halothane and nitrous oxide, based on a contemporary concern in the late 1970's of spontaneous abortion due to anaesthetic gases and technological considerations for operating theatre equipment at that time, especially the lack of scavenging equipment to remove waste anaesthetic gases. It was not based on the human and animal toxicity dataset for MOF now available.

As MOF is only used intermittently for short term analgesia one of the technical challenges in deriving an occupational exposure level (OEL) for it is the suitability of human data describing the effects at sub-therapeutic doses to identify a point of departure for the analysis. Although employed extensively since the 1960's the clinical data reported in the medical literature was generated to report safety, tolerability and efficacy at therapeutic (anaesthetic) doses. This paper describes the development of an OEL for brief exposure to lower doses of MOF to produce analgesia following a review of non-clinical toxicological, pharmacological and pharmacokinetic data along with clinical pharmacodynamic, pharmacokinetic and safety information and the results of controlled clinical trials as well as the pathogenesis of its principal toxic action on the kidney. The benchmark dose procedure has been employed to derive an OEL affording a considerable degree of protection for exposed workers. The procedure exemplifies how contemporary risk assessment models/approaches can be combined with data from clinical experience/clinical safety studies to derive a transparent dose response assessment (Silverman et al., 1999).

Given that the term OEL is associated with guidelines and standards the term is avoided in the abstract and the term MEL, maximum exposure level, is used within this paper.

This paper derives an MEL for MOF in the light of its odour as well as typical exposure conditions in occupational settings. A summary of exposure studies and a volatilization model has been used to estimate exposure in occupational (ambulance and treatment rooms) settings.

## 2. Metabolism and toxicokinetics

MOF has a very high blood to gas partition coefficient of 12 (Goodman and Gilman, 1980). This means it is readily transferred to blood during each respiratory cycle, little if any remains in the alveoli just before the next inhalation, and the time required for equilibrium to be reached is relatively longer than most other volatile gases. It also means that the elimination will be delayed due to partitioning to fat. Blood levels following inhalation plateau after less than 1 h of constant exposure (Cousins and Mazze, 1973).

Toxicokinetic information for MOF is available in human volunteers (n = 12) receiving MOF during anaesthesia (induced by vapouriser set a 1.5% during induction and 0.3–0.5% for maintenance during the three hour administration (Mazze et al., 1971). Rapid and extensive metabolism was reported with approximately 40->60% of the parent compound converted to metabolites and approximately 20% excreted unchanged in exhaled air.

Studies in human in vitro hepatic microsomal fractions have shown that metabolism of MOF is catalysed predominantly by cytochrome P450s. Given that it is inducible by phenobarbital and ethanol it is likely that a number of isoenzymes is involved in its metabolism including CYP 2B4, 2D and CYP2E1 (Zhang et al., 2005; van Dyke, 1973; Yin et al., 1995; Sweeney and Bromilow, 2006). In humans, CYP2E1 and perhaps 2A6 and 3A4 are present in the kidney and may be involved in the metabolism of MOF (Kharasch et al., 1995).

Metabolism occurs via two pathways (Kharasch et al., 2006a, b); oxidative demethylation and dehalogenation. MOF metabolism results in at least six excretable metabolites, carbon dioxide, fluoride, oxalic acid, dichloroacetic acid, difluoromethoxyacetic acid and chloride (Yoshimura et al., 1976). The main focus within this paper is the formation of fluoride metabolites given their importance to the mode of action for MOF toxicity.

To assess which strain of rat might serve as an appropriate model for MOF toxicity, adult male Fischer 344, Buffalo, Wistar, Sprague Dawley and Long-Evans rats were anaesthetised with 0.5% (5000 ppm) MOF for three hours. Fischer 344 and Buffalo rats metabolised MOF to a greater extent than the other three strains. Serum and urinary fluoride levels in Fischer 344 and Buffalo rats were greater than in the other strains of rats even though the blood concentrations of MOF was approximately the same in all strains. Since only Fischer 344 rats developed biochemical and pathological evidence of a renal lesion it was evident that kidney damage was not related to blood levels of MOF but to fluoride metabolites (Mazze et al., 1973).

Mazze et al. (1972) also investigated the MOF dose response in Fischer 344 rats for the purpose of delineating the renal toxicity of MOF. The authors divided 30 rats into five groups of six rats each. Concentrations and duration of exposure to MOF were increased in each of group 1 to 3 from 0.25% for 1.5 h to 0.75% for 6 h. Groups 4 and 5 served as control groups. Urinary fluoride (an excretion product of MOF metabolism) was measured on a daily basis for seven days post-anaesthesia. Peak urinary fluoride concentrations were reached on day three and amounted to approximately 6–8 times the preanaesthetic concentration of urinary fluoride.

Corbett & Ball. (1971) investigated excretion of MOF in end expired air of patients following prolonged anaesthesia with MOF and of the anaesthesiologists who administered the anaesthetic. Anaesthesiologists were exposed to MOF from anaesthetic equipment (Pentec-2-vaporiser with a gas flow of 5 L/minute), which at the time did not commonly employ scavenging systems (e.g. activated charcoal). MOF was detectable in end expired air from patients for 10–18 days after anaesthesia and in anaesthesiologists for up to 30 h after exposure. Anaesthesiologists were exposed to 1.3–9.8 ppm MOF.

The authors investigated the use of a 'gas trap' (a balloon fitted over the pop-off valve of the anaesthetic apparatus used and diversion of waste gas to an exhaust system to remove the gas from the room). The concentrations in the operating room air then ranged from 0.015 to 0.095 ppm (i.e. 97% efficiency in reducing environmental vapour concentrations). Only two anaesthesiologists participated in the study and exposures were 130 min, 390 min and 300 min. Duration of excretion was found to be related to the duration of exposure. Table 1 shows the relationship between duration of exposure and time taken to eliminate MOF to levels below the detection limit (0.01 ppm).

The anaesthesiologist with an exposure of 130 min had MOF detectable in end-tidal air for up to 10 h. The anaesthesiologist with an exposure duration of 390 min had an increase in urinary fluoride

**Table 1**
Methoxyflurane elimination following anaesthesiologist exposure (Adapted from Fig 4 – MOF decay curves of anaesthesiologists following administration of anaesthesia Corbett and Ball., 1971).

| Duration of exposure of anaesthesiologist to MOF (minutes) | Time for elimination of MOF in expired air (ie <0.01 ppm MOF) (minutes) | Ratio of time for elimination/Duration of exposure |
|---|---|---|
| 130 | 630 | 4.8 |
| 300 | 1620 | 5.4 |
| 390 | 1740 | 4.5 |

ion with a peak 5 h after exposure. The peak urinary fluoride concentration following a 390 min indoor air exposure to between 1.3 and 9.8 ppm occurred between 12 and 18 h from the beginning of the operation and was approximately 0.15 μM.

Strum et al., 1991 investigated the pharmacokinetics of simultaneous administration of halothane, isoflurane, enflurane and methoxyflurane (658 ppm) during 30 min of exposure to volunteers; including seven healthy young subjects: men (n = 3) and women (n = 4). Elimination occurred over 5–12 days. The study confirmed that the elimination of the anaesthetics via expired air occurred at very similar rates for the different anaesthetics measured. Expired concentrations of all the anaesthetics dropped to less than 10% of the original concentration in less than 1 h. After 2 days less than 0.1% of the inhaled concentration was detected in breath. The slower rate of elimination of the last 10% of MOF was attributed to the high solubility of MOF in lipid which results in partitioning to fat tissue followed by slow diffusion from these sites.

The results of both Corbett and Strum show that sub-anaesthetic concentrations of MOF are rapidly eliminated following inhalation and that most of the elimination occurred within several hours of the end of even prolonged exposure to high anaesthetic doses of MOF.

## 3. Hazard identification

### 3.1. CNS effects

The halogenated anaesthetics exhibit four sites of biological activity; central nervous system depression (amnesia, analgesia, respiratory depression), cardiovascular system (reduced cardiac output by 20%–50%, depression of contractility, and production of arrhythmias), liver dysfunction and nephrotoxicity (inhibits sodium chloride reabsorption in the thick ascending limb and inhibits ADH-mediated reabsorption of water, possibly due to disruption in adenylate cyclase). These effects occur at high concentrations (>1000 ppm) (Goodman and Gilman, 1980).

Hosick et al. (1971) investigated cerebral somatosensory potentials by measuring electroencephalogram traces while healthy human volunteers were exposed to sub-anaesthetic concentrations of cyclopropane, diethyl ether, methoxyflurane and enflurane. Volunteers were asked to respond to electrical stimuli and the responses recorded. The authors state that end-tidal concentrations of MOF were too low to measure by gas chromatography (0.008%, 80 ppm detection limit). The inspired concentrations of MOF were up to 0.38% (3800 ppm) for a duration of up to 2 h. One subject who received 0.38% for 1 h lost the ability to cooperate at this concentration. Exposure to MOF was ceased and when the subject was alert, calm and relaxed he was retested. At this time end-expired air concentrations could not be measured (below 80 ppm limit of detection). In low concentrations MOF was found to suppress evoked potentials with latencies greater than 50 msec; early responses were unaffected but late response was suppressed and then returned after further recovery. The subject had difficulty counting and concentrating but was able to recall all phases of the study. Four of the six subjects reported were amnesic for part or all of the study.

Swann et al. exposed mice to various concentrations of MOF and measured respiratory rate, depth and configuration while the animals were inhaling MOF. The concentrations used were 1000, 2000, 4000, 8000, 16,000, 32,000, 64,000, and 128,000 ppm. Exposures were for five minutes. Sporadic body movements but not anaesthesia were noted at 1000 ppm with light anaesthesia at 4000 ppm and deep anaesthesia with irregular respiration at 32,000 ppm.

In a pilot telemetric evaluation in two beagle dogs exposed to MOF by inhalation, arterial blood pressure (systolic, diastolic and derived mean), heart rate (derived from the blood pressure waveform), respiration (rate, tidal volume and derived respiratory minute volume (RMV)) and EEG were monitored. The dogs were dosed by inhalation for 10–15 min at 0.01, 0.02, 0.04, 0.08, 0.1 and 0.5% (i.e. approx. 100, 200, 400, 800, 1000 and 5000 ppm). Behavioural and clinical observations were conducted predose and at the end of each dose level exposure, then at approximately 0.5, 1, 2 and 4 h. No adverse effects (clinical or behavioural signs, arterial blood pressure (systolic, diastolic or mean), heart rate or respiratory parameters (rate, tidal volume or minute volume) were noted at concentrations up to 0.1% for 15 min. At 0.5% for 10 min MOF produced an increase in heart rate, an increase in respiration rate, a decrease in tidal volume and an increase in minute volume (Huntingdon Life Sciences, 2013).

In a developmental toxicity study (Pope et al., 1978) pregnant Swiss mice were exposed to either 2 or 60 ppm MOF for 4 h per day and for 9 days (gestation days 6–15). The authors noted that there were no adverse behavioural effects and no changes to body weight gain for exposed dams.

Similarly Sprague-Dawley pregnant rats were exposed for 8 h/day for 20 days (gestation day 1–21) at 100, 400, and 800 ppm MOF. The authors did not comment on behavioural effects at 100 or 400 ppm but did note that the maximum concentration was selected as the 'maximum tolerated sub-anaesthetic dose' and that some animals were somnolent at 800 ppm. No other adverse effects in the dams were noted (Pope et al., 1978).

Overall the available studies indicate that the acute effects occur at relatively high concentrations and do not include respiratory system effects such as respiratory irritation.

### 3.2. Kidney effects

The kidney toxicity of MOF has been thoroughly investigated in animals and humans (Kharasch et al., 1995; Kenna and Jones, 1995; Mazze et al., 1972, 1973; and 1974). The renal injury caused by MOF is characterised by acute renal failure with accompanying serum hyperosmolarity, hypernatremia, urinary hypoosmolarity, and vassopressin resistant polyuria due to proximal tubular damage. These changes are associated with the inhibitory effects of the metabolite fluoride on solute and water reabsorption (Schnellmann, 2013; Hoffman et al., 2002). Fluoride inhibits sodium chloride reabsorption in the thick ascending limb and inhibits ADH-mediated reabsorption of water, possibly due to inhibition of adenylate cyclase (Mazze et al., 1973; Schnellmann, 2013; Hoffman

et al., 2002).

Clinical investigations have found the severity of kidney damage to be proportional to the dose of MOF administered to patients (Cousins and Mazze, 1973). Mazze et al. (1972, 1973, and 1974) were able to reproduce dose-related MOF nephrotoxicity in the F344 strain of rat and to demonstrate the cardinal importance of fluoride in the pathogenesis of the renal lesion.

Prospective clinical studies have indicated the threshold peak serum concentration of fluoride above which kidney damage occurs in humans is 40 μmol/litre as produced after a dose of 2.0 MAC (minimal alveolar concentration) hours (approx. equivalent to 192,000 ppm min) (Cousins and Mazze, 1973). Thus fluoride production is proportional to the inhaled concentration of MOF and duration of exposure time.

Two anaesthesiologists were exposed to 1.3–9.8 ppm MOF for 130 min, 390 min or 300 min (Corbett and Ball, 1971). Duration of excretion of fluoride was found to be related to the duration of exposure. The mean urinary fluoride concentration following a 390 min exposure of between 1.3 and 9.8 ppm was 0.15 μmol with a peak occurring approximately 5.5–8.5 h after exposure.

### 3.3. Liver effects

Severe liver toxicity following MOF exposure during anaesthesia and analgesia has been observed on very few occasions. It is described as an “extremely rare” adverse reaction characterised by clinical and histological features that closely resemble those of halothane hepatitis. It is postulated that the mechanism is mediated by the immune system perhaps following direct toxicity of reactive metabolites (Kenna and Jones, 1995).

Adaptive changes in biochemical markers and liver function adverse effects have been reported in animal toxicity studies following single or repeat exposure. Although the data available for this endpoint is limited adverse effects have only been observed at high concentrations.

No renal effects were reported in male and female Wistar rats, Guinea pigs or rabbits exposed to a sub-anaesthetic dose of 200 ppm (0.02%) MOF in air for 7 h/day, 5 days per week and a total period of 7 weeks (Chenoweth et al., 1972; equivalent to 84,000 ppm min). Hepatic changes were observed, consisting of minimal focal fatty metamorphosis in rats, minimal to marked centrilobular fatty metamorphosis in Guinea pigs, and minimal centrilobular fatty metamorphosis in rabbits. In the latter species, hepatic findings were occasionally accompanied by elevated serum levels of alanine aminotransferase (ALT) and aspartate aminotransferase (AST).

In 12 male (Fischer 344) rats, continuously exposed to MOF vapour at a sub-anaesthetic dose of 50 ppm for a period of 14 weeks (equivalent to 6,480,000 ppm min), reduced body weights were observed at the end of the treatment period (approximately 60% decrease in comparison to controls) (Plummer et al., 1985). MOF exposure caused an increase of water intake (15–20%) and urinary volume (indicating nephrotoxicity). These effects were relatively mild, were not accompanied by kidney histopathological changes, and were fully reversible after a 4-week recovery period. Serum alanine amino transferase ALT was markedly enhanced and liver weight relative to body weight was increased. Hepatic cytochrome P450 levels were significantly reduced. Microscopically, the liver showed a variable extent of fatty metamorphosis, and foci of hepatocellular degeneration and necrosis. After 4 weeks without treatment, serum ALT levels had decreased considerably (but not to control levels), and fat was no longer observed in the liver. Foci of hepatocellular degeneration and necrosis were however still present.

### 3.4. Genotoxicity and carcinogenicity

MOF was negative for genotoxicity in both an in vitro bacterial mutagenicity and in vivo mammalian micronucleus assay.

In a recently conducted (Williams, 2009) Ames test according to GLP and OECD TG 471, it was concluded that MOF did not induce mutation in five histidine-requiring strains (TA98, TA100, TA1535, TA1537 and TA102) of *Salmonella typhimurium* when tested in the plate incorporation assay. Tests occurred at concentrations up to 5000 μg/plate in the absence and presence of a rat liver metabolic activation system (S-9).

In an in vivo GLP micronucleus test (OECD TG 474) MOF was administered to Sprague-Dawley CD rats intravenously (25, 50, 100 mg/kg) by bolus injection in the lateral tail vein. No statistically significant increases in the frequency of micronucleated immature erythrocytes and no substantial decreases in the proportion of immature erythrocytes were observed in rats treated with MOF at any treatment level, compared to vehicle control values (Pritchard, 2006).

The International Agency for Research on Cancer (IARC) evaluated general evidence about the carcinogenicity of volatile anaesthetics in 1976 and concluded that there was inadequate evidence of carcinogenicity to animals or humans (Group 3). The conclusion was confirmed following review in 1987 (IARC, 1987).

### 3.5. Developmental and reproductive effects

The developmental toxicity of trace, sub-anaesthetic and anaesthetic exposures to methoxyflurane was examined in Swiss/ICR mice (Wharton et al., 1980). No adverse effects on reproduction or foetal development were demonstrated following exposure to trace (2 ppm) and sub-anaesthetic (60 ppm) concentrations of methoxyflurane for 4 h daily on days 6 through 15 of pregnancy. Exposure to an anaesthetic concentration (2000 ppm; 0.2%) for the same period resulted in decreased foetal weight, decreased ossification, and delayed renal maturation. Additionally, the incidence of minor skeletal anomalies was increased. It is concluded that gestational exposure of mice to trace or sub-anaesthetic concentrations of methoxyflurane does not result in reproductive loss or morphologic abnormalities in their offspring. The highest NOEL in this study was 60 ppm.

Mazze et al. (1986) investigated the reproductive and developmental toxicity of halothane, isoflurane, enflurane and nitrous oxide. Sprague Dawley rats were exposed to 0.8% (800 ppm) halothane, 1.05% isoflurane, 1.65% enflurane and 75% nitrous oxide on pregnancy days 14–16, 11–13, or 8–10. Exposure resulted in light anaesthesia. Reproductive indices were determined and 5178 offspring were examined for external, internal and skeletal abnormalities. There were no major or minor teratologic effects in anaesthetic treated groups, although several developmental variants were observed in halothane and enflurane treated groups. Nitrous oxide exposure on days 14–16 resulted in a three-fold increase in foetal resorptions. The results suggest that the volatile halogenated anaesthetics are not teratogenic.

An abstract by Corbett et al. (1974) reported a study where pregnant Sprague-Dawley rats were exposed to either 10 or 100 ppm MOF from days 8–15 of pregnancy for 8 h per day. The authors report that there were no differences between experimental and control groups in number of implantations per rat, resorptions, foetal anomalies, sex ratio, average foetal crown-rump length, or average foetal weight. The veracity of these findings cannot be confirmed as the authors have not published a full account of the study in a peer reviewed journal. However the results are consistent with published studies by Pope et al. (1978), Wharton et al.(1980), Mazze et al. (1986).

Wharton et al. (1978) assessed fertility, reproduction and postnatal survival in mice chronically exposed to halothane at exposures of between 0.05% for 0.5 h/day (h/d), 0.05% for 2 h/d, 0.1% for 4 h/d, 0.3% for 4 h/d, 1.0% for 4 h/d. Exposures to 0.1% (1000 ppm) or higher resulted in decreased maternal weight gain, decreased foetal length and weight and early postnatal weight gain. No adverse effects on reproduction were noted at the two lowest exposure levels studied. Pregnancy rate, implantation rate and number of live foetuses per litter were decreased at 0.3% (3000 ppm) halothane. The NOEL for halothane in this study was 0.05% (500 ppm).

Epididymal spermatozoa of mice were examined for morphological abnormalities following exposure to 0.1 MAC or higher concentrations of anaesthetics (MOF (0.01 and 0.1%, 100–1000 ppm) for 4 h per day for 5 days (20 exposure hours). Twenty eight days after exposure epididymal spermatozoa were examined. No changes in the percentages of abnormal spermatozoa were found in either MOF exposure group (Land et al., 1981). A number of reviewers have commented on exposure to anaesthetic gases and reproductive/developmental outcome (Boivin, 1997; Tannenbaum and Goldberg, 1985; Ferstandig, 1978; NIOSH, 1977; and NH&MRC, 1977). Furthermore a number of studies have investigated MOF (or other volatile anaesthetics) for reproductive and developmental toxicity in rats and mice (Pope et al., 1978, Wharton et al., 1980, Mazze et al., 1986, Wharton et al., 1978, Land et al., 1981). Some reviews of occupational risks due to exposure to anaesthetic gases (noting that many of these did not include assessment of MOF exposure) suggest an increased risk of spontaneous abortion (Boivin, 1997; NIOSH, 1977 and NH&MRC, 1977).

Animal studies investigating the reproductive and developmental toxicity of MOF consistently show that MOF is neither teratogenic nor causes reproductive effects at sub-anaesthetic exposures at non-maternally toxic doses.

Boivin (1997) conducted a meta-analysis of published epidemiological studies investigating the association between maternal occupational exposure to anaesthetic gases and risk of spontaneous abortion. The overall relative risk was 1.48 (95% confidence interval [95%CI], 1.4 to 1.58). Utilising only those studies rated by the authors as rigorous the estimate of risk increased to 1.9 (95% CI: 1.72 to 2.09). They conclude that data obtained in the era where prescavenging of anaesthetic gases was not performed indicate an increased risk of spontaneous abortion. The most commonly used anaesthetics within the study period (studies published between 1971 and 1995) were halothane and nitrous oxide. The study does not include analysis of causal relationships with any particular anaesthetic. It is unclear whether the studies controlled for other risk factors such as ionising radiation (e.g. dentistry and veterinary practices) and pesticide exposure (veterinary practices). It is noted that many of the reviewed studies had deficiencies and where these could be identified they were subjectively weighted by the authors.

Tannenbaum and Goldberg (1985) reviewed the epidemiological literature concerning occupational exposure to trace concentrations of anaesthetic gases and reproductive outcomes. They comment that a variety of evidence suggests that chronic exposure to incidental low doses of anaesthetic gases, as occurs in the occupational setting, is a risk factor for spontaneous abortion and congenital defects. The most commonly used anaesthetics within the study period (studies published between 1971 and 1995) were halothane and nitrous oxide. The authors note that there was a large variability in levels of gas concentrations in operating theatres. For instance studies from the 1970s reported nitrous oxide levels ranging from 130 to 7000 ppm in the anaesthesiologists breathing zone. The study does not include analysis of causal relationships with any particular anaesthetic. However there are inconsistencies between studies, arising primarily from investigational methodologic problems. For example, lack of criteria for exposure or outcome, poor survey response rates, selection bias, lack of validation of outcome, recall bias and lack of control of potentially confounding variables. The authors state "*results from the majority of studies do suggest that there is no increase in spontaneous abortions amongst wives of exposed male personnel and that there is no increase in birth defects among the offspring of exposed parents*".

Ferstandig (1978) reviewed the available animal and human toxicity data for reproductive effects of inhalation anaesthetics. He concluded that "*short exposures to even high concentrations of inhalation anaesthetics has either no adverse reproductive effects or has statistically questionable effects*".

Pope et al. (1978) investigated fetotoxicity in rats following chronic exposure to halothane, nitrous oxide or methoxyflurane. Pregnant Sprague Dawley rats were exposed for 8 h a day throughout the 21 days of gestation to MOF 0.01%, 0.04% or 0.08%, (100–800 ppm). Foetal loss in the MOF groups was unchanged compared to controls and no gross skeletal anomaly related to exposure was seen, but there was a dose related decrease in foetal weights, size and development as evidenced by slight skeletal variations in the ossification centres of the metacarpals and phalanges. The authors state that dams exposed to the highest concentrations were drowsy during exposure. Based on decreased foetal weight a no effect level (NOEL) was not identified in this study. The authors postulate that the effects observed are a general effect of the anaesthetic on the nutritional status of mother rather than a direct effect on the foetus.

In summary the animal reproductive studies are negative and human clinical and epidemiological findings are insufficient to conclude a causal relationship.

## 4. Risk characterisation

The typical practice in developing occupational exposure levels is to identify an effect level or concentration for the most sensitive relevant adverse effect as a "point of departure" (POD) and then apply factors to address uncertainties in extrapolation from the identified effect levels. The present health risk assessment includes an analysis of the dose-response relationship between occupational exposure to methoxyflurane and health related outcomes using the benchmark dose modelling approach (BMD) (US EPA, 2012; WHO, 2009; EFSA, 2009). In the case of MOF renal toxicity was considered to be the critical and most sensitive adverse effect.

To determine the point of departure, benchmark concentration (BMC) modelling was conducted to estimate a concentration associated with a 10% extra risk (the $BMC_{10}$) of subclinical kidney toxicity (of minimal severity or greater) and a 95% lower confidence bound concentration estimate (the $BMCL_{10}$). The benchmark response level (BMR) is defined as a specified increase in incidence over background. A BMR of 10% (extra risk) is used as a default for quantal data sets (US EPA, 2012; EFSA, 2009) as it is roughly equivalent to the experimental no observed adverse effect level (NOAEL). The BMC modelling to derive the POD was conducted using US EPA software BMDS. Uncertainty factors were applied to the POD to derive the maximum exposure level (MEL) expressed as an 8 h time weighted average.

### 4.1. Selection of critical effect

The key organs associated with MOF toxicity in anaesthetic doses include; central nervous system (amnesia, analgesia, anaesthesia, respiratory depression), cardiovascular system (reduced cardiac output by 20%–50%, depression of contractility, and production of arrhythmias), liver dysfunction and nephrotoxicity (due

to tubular damage caused by the metabolite, fluoride).

Both the central nervous system and cardiovascular effects are related to exposure to high concentrations.

Methoxyflurane induced liver toxicity in humans has been a very rare phenomenon after analgesic or anaesthetic exposures. Although incidental exposure to MOF has been reported to result in small transient increases in biochemical markers of liver function, the changes are within or only just outside of normal population ranges and are therefore not indicative of liver damage. On repeat exposure (14 weeks at 50 ppm for 1 h per day) animal study did show direct liver toxicity can occur. It is noted that these effects co-occurred with serum inorganic fluoride concentrations above 50 μM/L (Plummer et al., 1985).

Kidney toxicity in both animal models and humans is dose-related, enhanced by enzyme induction and attenuated by enzyme inhibition. The same pattern of renal damage can be induced in animals not exposed to MOF by the injection of inorganic fluoride (Cousins and Mazze, 1973; Mazze et al., 1974; Mazze et al., 1972).

In a carefully designed and controlled clinical study, Cousins and Mazze (1973) showed that the onset of sub-clinical toxicity in people did not occur until exposure to MOF exceeded the total dosage of 2.5 MAC hours, corresponding to a serum inorganic fluoride concentration of 50 μM/L.

In a recent review (Dayan, 2016) of laboratory and clinical data relevant to nephrotoxicity and methoxyflurane use in analgesia (since the mid-1960's) the authors concluded that analgesic use of methoxyflurane was not associated with overt evidence of renal dysfunction in adults or children. The duration of exposure ranged from about 6 to 8 min to 40–60 min or more in prehospital and accident and emergency use as well as obstetrics, brief orthopaedic and dental procedures and wound dressings. The total number of healthy subjects and patients amounted to several thousand adults and children down to the age of 1 year.

Kidney toxicity was selected as the critical effect because it is the predominant effect observed both on single exposure to high concentrations and after repeat exposure to lower concentrations.

### 4.2. Selection of key study

Cousins & Mazze. (1973) investigated the dose-response relationship between MOF and nephrotoxicity in men. This study was chosen as the key study as it was the most robust clinical investigation of MOF and kidney toxicity.

The study population consisted of 26 volunteers, all healthy men scheduled for elective surgery; their ages and weights are not disclosed. 18 participants received MOF in varying concentrations and 8 participants, making up the control group, received halothane.

Anaesthesia was induced with thiopental sodium followed by succinylcholine chloride and maintained with MOF (or halothane; not discussed here). The MOF (or halothane) was delivered via inhaler connected to an anaesthetic vaporiser at a gas flow rate of 6 L/min (for a mean duration of 3.8 ± 0.5 h); MOF (or halothane) was supplemented with 50% nitrous oxide and 50% oxygen.

The study population were divided into four dose groups as per Table 2.

MAC is defined as the minimum alveolar concentration to produce surgical anaesthesia in 50% of healthy patients. According to Cousins and Mazze (1973), 1.0 MAC methoxyflurane is equivalent to 0.16 vol % (1600 ppm).

Exposure duration expressed as MAC-hours ranged from 1 to 9 MAC-hours. MAC-hours represent the minimum alveolar concentration (%) multiplied by the duration of anaesthesia (h).

The mean exposure duration was 3.8 ± 0.5 h. For Group 2 participants (0.5 MAC) exposure was approximately 2.0 MAC-hours (3.8 h x 0.5 MAC = 1.9 MAC-hour).

**Table 2**
Study dose groups.

| Study population grouping | Dose |
|---|---|
| 1[a] | 1.0–1.5 MAC halothane |
| 2 | 0.5 MAC methoxyflurane |
| 3 | 1.5 MAC methoxyflurane |
| 4 | 1.0 MAC methoxyflurane |

[a] Postoperatively, participants who received halothane displayed symptoms of mild hyponatremia and serum hypoosmolality, with a prompt return to preoperative urine concentrating ability. These symptoms were considered normal for the control group. Group 1 participants are not discussed further.

Serum fluoride levels were monitored in this study as a dose-dependent marker of the effect of methoxyflurane. Each volunteer was also monitored for signs of nephrotoxicity classified as: subclinical, mild clinical and clinical toxicity. Subclinical toxicity was characterised by delayed return to maximum preoperative urine osmolality, unresponsiveness to vasopressin administration, and elevated serum uric acid concentration. In addition to subclinical symptoms, study participants with mild clinical toxicity were reported to have serum hyperosmolality, hypernatremia, polyuria, and low urine osmolality (306–372 mOsm/kg). Those with symptoms of clinical toxicity had more pronounced serum and urine abnormalities and a reduction in creatinine clearance. These effects were reversible.

### 4.3. Modelling the point of departure

Cousins and Mazze (1973) plotted their findings for each volunteer with respect to dose and serum fluoride levels and the presentation of symptoms. Individual participant data were only provided in graphed form so graphing software was utilised to transform the information into numerical form for further processing. This was done by reverse engineering each axis and data point into a new graph by assigning coordinates to each data point.

Once numerical data were extracted they were converted from MAC-hours to parts per million concentration in time using the following equation:

concentration (ppm) x time (min) = ppm.min

1 MAC-hour is approximately 96,000 ppm x minutes (ppm.min) calculated as 1600 ppm (1 MAC) x 60 min. The extrapolated data are provided in Table 3 and Fig. 1.

The critical effect level (the mean threshold for toxicity) described by Cousins & Mazze was found to be approximately 2.5 MAC-hours. Subclinical toxicity was found to occur at exposures 2.5–3.0 MAC-hours which resulted in peak serum fluoride ion concentration ranging from 50 to 80 μM. There was no evidence of renal damage after exposure to 2 MAC-hours. Patients receiving 2.0 MAC-hours or less methoxyflurane had peak serum fluoride ion concentrations <40 μM which was not associated with nephrotoxicity.

### 4.4. Benchmark concentration derivation

Data from Fig. 1 was split into dose groups in order to plot incidence vs dose using US EPA Benchmark Dose Software (BMDS Wizard v1.8). The dose-response data was grouped into dose ranges (5 dose groupings) as indicated in Fig. 2. The dose range grouping considers five distinct groups with regard to observed effects at ranging concentrations. The grouping of data is important for

**Table 3**
Extrapolated[a] Dose-response data from Cousins and Mazze (1973).

| Study group | X (MAC hours) | X (ppm min) | Y (fluoride conc. μM) |
|---|---|---|---|
| MAC "0.5" Group Data | 1.5 | 144,000 | 35 |
| | 2 | 192,000 | 37 |
| | 2.5 | 240,000 | 36 |
| | 2.75 | 264,000 | 31 |
| | 3 | 288,000 | 64 |
| MAC "1" Group Data | 1 | 96,000 | 23 |
| | 2 | 192,000 | 30 |
| | 2.5 | 240,000 | 50 |
| | 3 | 288,000 | 72.5 |
| | 3 | 288,000 | 77.5 |
| | 2.5 | 240,000 | 94 |
| | 7 | 672,000 | 117 |
| | 9 | 864,000 | 175 |
| MAC "1.5" Group Data | 2.75 | 264,000 | 41 |
| | 4.5 | 432,000 | 97 |
| | 5 | 480,000 | 90 |
| | 5 | 480,000 | 116 |
| | 9 | 864,000 | 80 |

[a] Data from *Peak serum inorganic fluoride concentration (F-) and degree of nephrotoxicity* Fig. 1, was extrapolated using graphing software (DataThief III).

benchmarking to achieve a dose-response curve with good fit and confidence in the model predictions.

Dose-response grouped data were plotted (dose vs incidence rate) using US EPA Benchmark Dose Software, BMDS Wizard.

The POD used is based on benchmark dose modelling. The benchmark dose method involves fitting mathematical models to dose-response data and using the different results (from the models employed) to select or derive a BMC that is associated with the predetermined benchmark response (critical effect). Because the individual statistical curve fits are approximations only the average POD from each of these statistical extrapolations is calculated and selected as the POD. Not all statistical extrapolations are used. Only those that meet statistical test criteria for goodness of fit. The average of eight models meeting minimum requirements for goodness of fit was used to derive the POD (30,404 ppm min). The modelled dose-response results (for eight mathematical models) are provided in Fig. 3 and are tabulated in Table 4. The lower confidence limit of the benchmark concentration is referred to as the BMCL.

### 4.5. Selection of uncertainty factors

The uncertainty factors considered and selected are presented in Table 4. The total uncertainty factor evaluates the available data from four aspects: human variability (kinetic and dynamic), adequacy of the database, and the use of a non-chronic study for selection of the POD. Interspecies extrapolation was not considered as human data were employed to derive the POD.

Occupational assessments based on human data typically apply a factor of 1–3 depending on the robustness of the data (Maier et al., 2010). For MOF renal toxicity has been observed in several different studies involving several different species (including humans) and the mode of action is well understood. The NOAEL for clinical renal toxicity has been defined with reasonable confidence.

Repeat exposure animal toxicity studies are available for critical endpoints for occupational exposure standard development. Developmental toxicity was investigated following exposure to trace (2 ppm) and sub-anaesthetic (60 ppm) concentrations for 4 h a day on gestation days 6 through to 15. The NOEL for this study was 60 ppm (i.e. higher than the POD). At higher concentrations in repeat exposure studies with rats (Wistar and Fischer 344), guinea pigs or rabbits resulted in evidence of liver toxicity. These results are discussed in Table 9 and Section 5.2.

The variability of metabolism of toxic chemicals caused by

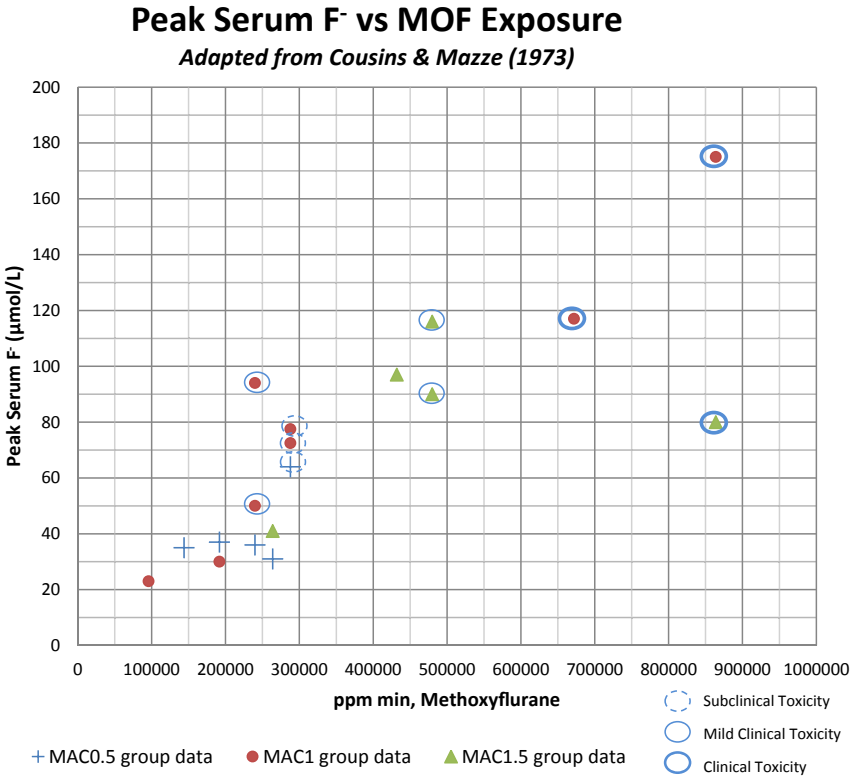



**Fig. 1.** Dose response data adapted from Cousins and Mazze (1973).

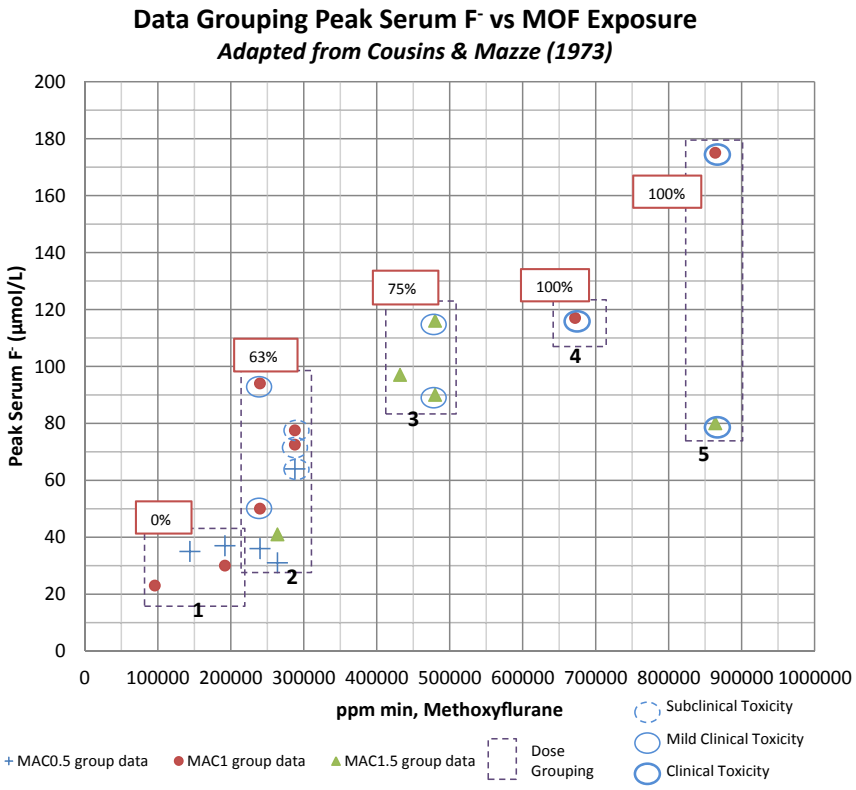



**Fig. 2.** Dose-response data grouping. [1] The maximum dose (192,000 ppm min MOF) selected for benchmarking as there were no incidences recorded for Dose group 1. [2] Conservatively the lowest dose concentration (240,000 ppm min MOF) used for benchmarking for Dose group 2. [3] Conservatively the lowest dose concentration (432,000 ppm min MOF) used for benchmarking for Dose Group 3. [4] One dose in this grouping, 672,000 ppm min MOF used for benchmarking for Dose group 4. [5] One dose in this grouping, 864,000 ppm min MOF used for benchmarking for Dose group 5.

genetic polymorphisms is expected to be captured by the pathway-related factors for general healthy adults (Pohl and Scinicariello, 2011). Dorne et al. (2005) include factors for CYP2E1 enzymes of 1.5 and 1.8 for the 95th and 99th percentile of the population, respectively (as compared to the median value).

Given that the assessment is for workers (a relatively healthy subpopulation of adults) and the critical effect is well characterised in human studies, the total uncertainty factor of 3 was selected.

### 4.6. Derivation of the maximum exposure level (MEL)

The derivation of the MEL follows conventional risk assessment methodology using the benchmark dose modelling approach. As previously described, benchmark dose software was used to model the dose-response relationship between MOF and the critical effect (based on the findings of the key study). The POD was derived from the average of the modelled BMCL (30,404 ppm min).

The derivation is intended as an eight hour time weighted exposure thus adjustment for averaging time was made. The 8-hr POD expressed as an 8 h average is 63 ppm.

An uncertainty factor of 3 was applied to the 8-hr POD to derive the MEL due to human toxicodynamic variability. Table 4 summarises the steps and decisions made to derive the occupational maximum exposure level. This includes the goodness of fit (GOF) evaluation for each of the BMC models utilised. All models exceed the US EPA (2012) recommended cutoff p-value of 0.1 and have a small Akaike's Information Criterion (AIC) value. Taken together with the visual inspection of curve fit, the average $BMC_{10}$ and $BMCL_{10}$ was used as the POD. The resulting 8 h MEL is 15 ppm.

The no observed adverse effect level in the key study was between 96,000 ppm min and 264,000 ppm min or expressed as an 8 h concentration, 200 ppm and 550 ppm. Thus the BMD is between 3.2 and 8.5 times lower than the NOAEL observed in the study. The derivation of an MEL using the NOAEL approach would result in an MEL of between 66 and 183 ppm assuming the same uncertainty factor of 3. The factor between the NOAEL and the BMD provides additional confidence that the uncertainty factor selected is appropriate.

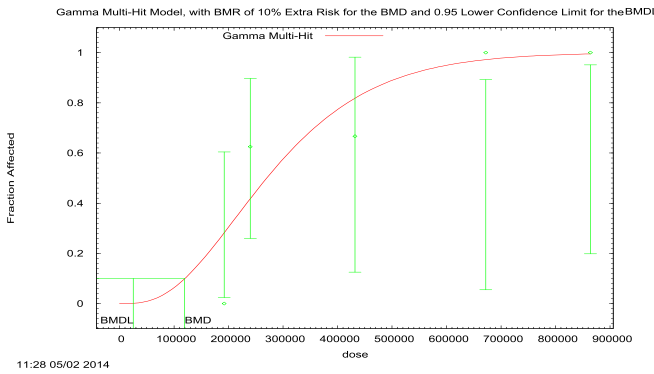
The BMD approach is generally considered reliable and preferable where data sets include two or more dose groups given that the entire dose response curve is utilised and the assessment is not reliant on any biases in dose selection.

## 5. Discussion

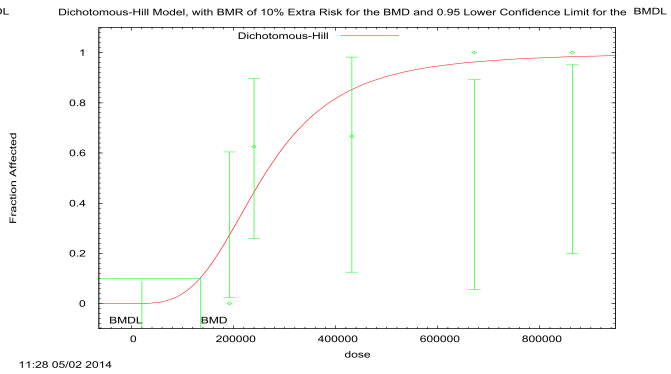
### 5.1. Consideration of exposure

#### 5.1.1. Monitoring data

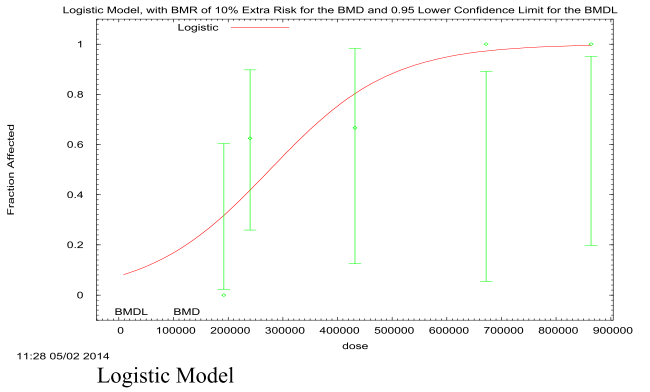
Methoxyflurane may be administered within an ambulance's patient compartment. The relatively small size of the compartment and the volatile nature of methoxyflurane indicate that occupational exposure to ambulance officers can occur. Ambulance officers have reported odour (often described as characteristic or fruity) during its administration. The frequency of occupational exposure will vary depending on the frequency of indicated uses during a day, the duration of patient inhalation, ventilation at the site of use and other administrative controls (trip duration and number in a working period). Given these variables, the magnitude of exposure is best considered through occupational exposure monitoring.



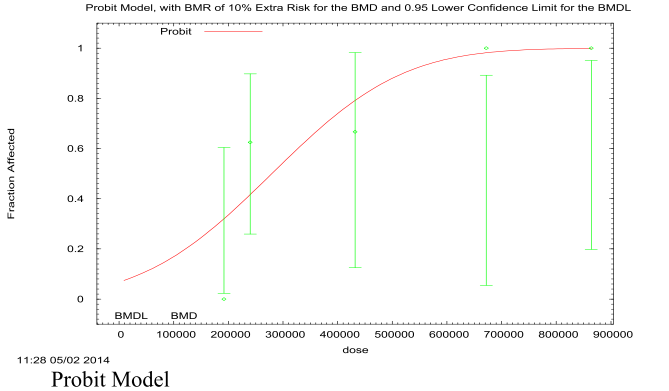

Gamma Model



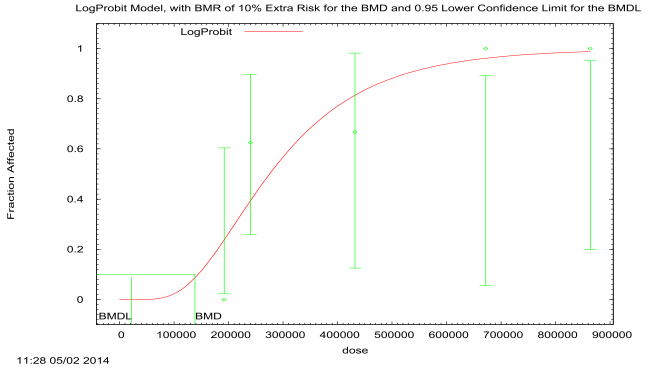

Dichotomous Hill Model



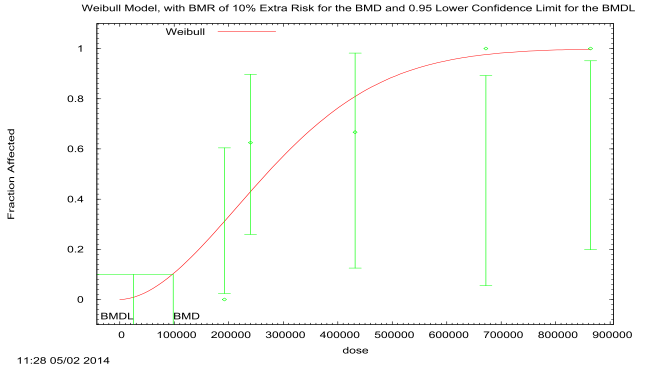

Logistic Model



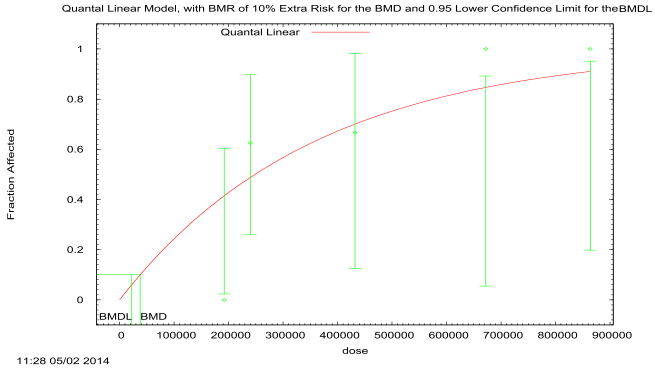

Probit Model



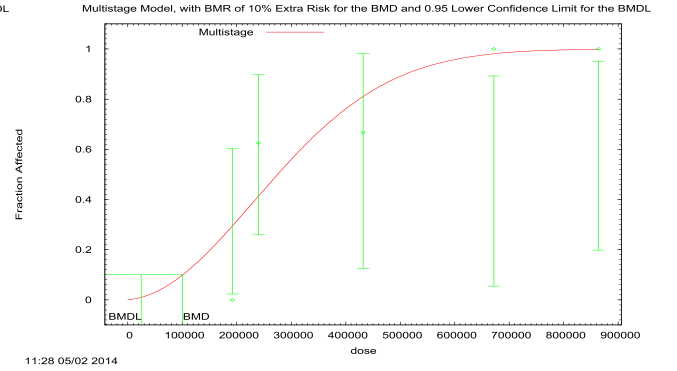

LogProbit Model




Weibull Model




Quantal Linear Model




Multistage 3° Model

**Fig. 3.** Benchmark dose modelling results (using US EPA BMDS Wizard).

**Table 4**
Summary of derivation of maximum exposure level (MEL).

| Model[a] | Goodness of fit | | $BMC_{10}$ (ppm min) | $BMCL_{10}$ (ppm min) | Ratio BMC/BMCL |
|---|---|---|---|---|---|
| | *p*-value | AIC | | | |
| Gamma | 0.324 | 22.908 | 1.2E+05 | 25074 | 4.79 |
| Dichotomous-Hill LogLogistic | 0.324 | 22.802 | 1.4E+05 | 20455 | 6.84 |
| Logistic | 0.301 | 23.229 | 99591 | 52483 | 1.90 |
| Probit | 0.305 | 23.193 | 94306 | 51653 | 1.83 |
| LogProbit | 0.332 | 22.759 | 1.4E+05 | 21779 | 6.43 |
| Weibull | 0.323 | 23.009 | 98004 | 24772 | 3.96 |
| Quantal-Linear | 0.430 | 22.020 | 37760 | 22320 | 1.69 |
| Multistage 3°[a] | 0.312 | 23.035 | 1.0E+05 | 24699 | 4.05 |
| Multistage 2° | | | | | |
| POD (average of all modelled BMCL10) expressed as ppm min | | | 103,708 | 30,404 | – |
| POD expressed as 8 h average | | | 216 | 63 | |
| Selection of POD | | | | 63 | |
| **Uncertainty factor selection** | | | | | |
| Interspecies | | | NA | NA | |
| Human variability – toxicokinetics | | | | 1 | |
| Human variability – toxicodynamics | | | | 3 | |
| Adequacy of database | | | | 1 | |
| Less than working life | | | | 1 | |
| MEL ppm | | | | 15 | |

Shaded cell is the selected POD used for derivation of the MEL.
[a] For the Multistage 3° model, the beta coefficient estimates were 0 (boundary of parameters space). The models in this row reduced to the Multistage 2° model.

Occupational monitoring has been conducted in the past (Hibbs and Gately (1999) and Coffey (2011)).

Hibbs and Gately (1999) measured MOF exposure of Ambulance Officers (AO's) in New South Wales (NSW) Australia during typical exposure conditions. During a two month period 302 samples (152 from Treating Officers and 150 samples from Drivers) were collected and assayed for MOF. Exposure estimation was performed by the collection of breathing zone air samples using passive dosimeters. The sampling rate allowed for measurement of exposures as low as 0.2 ppm for less than 1 h. The ambulance stations chosen were selected based on their previous high MOF usage.

The 8 h TWA exposure was calculated by Hibbs and Gately (1999) and presented in Table 5. The exposures are well below the derived MEL of 15 ppm (8 h TWA).

Duration of exposure and magnitude of exposure were not related indicating that the exposures to MOF were acute and intermittent. The vast majority of AO's were exposed only once per day and the mean exposure period was 21 min.

Coffey (2011) measured MOF exposure of AO's from the St John Ambulance Service in Western Australia during typical exposure conditions. During a one month period a total of 45 samples were collected on Ambulance Attendants, 45 on Ambulance Drivers and 44 in treatment areas. Exposure estimation was performed by the collection of breathing zone (attendants and drivers) air samples using passive dosimeters (3 M organic vapour monitoring (OVM) badges). The samples were collected during the event defined as the duration of exposure during treatment. The duration of exposure ranged between 15 min and 53 min (mean 30 min). The average 8-h TWA for attendants and drivers are 0.23 and 0.15 ppm respectively. The breathing zone concentrations ranged between 0.2 ppm and 7 ppm per treatment. The maximum 8-h TWA was estimated at 1.5 ppm. The vast majority of AO's were exposed only once per day and the mean exposure period was 30 min. Expressed either as an exposure per treatment or as an 8 h time weighted average the breathing zone concentrations are all well below the derived MEL of 15 ppm (TWA).

One of the uncertainties in the derivation of the MEL is the potential accumulation of serum fluoride on repeat exposure to MOF over a working week. The relationship between serum fluoride and MOF inhalation exposure from Cousins and Mazze (1973) is presented in Fig. 4. The line of best fit was explored for this dataset and is presented in Fig. 3. Using the equation derived from the line of best fit, the serum fluoride concentrations at the MEL and 99 breathing zone exposures reported by Coffey (2011) are plotted into Fig. 4. The estimated serum fluoride concentrations at the MEL and at the maximum ambulance officer exposure are 112 and 285 times lower than the threshold for kidney toxicity of 40 μM. The maximum ambulance officer exposures are likely to reflect the peak serum fluoride concentrations of occupationally exposed individuals. The estimates provide additional confidence in the approach taken to derive the MEL.

Both the results of Corbett & Ball. (1971) and Strum et al. (1991) show that sub-anaesthetic concentrations of MOF are rapidly eliminated following inhalation with most of the elimination occurring within several hours. Using the mean exposure time reported in Coffey (2011) and the Ratio of Time for Elimination/Duration of Exposure presented in Table 1 from an analysis of Corbett & Ball. (1971), the time to elimination is anticipated to be within one working day even if repeat intermittent exposure occurred. The patients exposed to MOF in Cousins and Mazze (1973) were exposed over an average of 4 h, a relevant timeframe for the present assessment.

**Table 5**
Average MOF exposure of ambulance officers (Hibbs and Gately, 1999).

| Exposure | Driver | Training officer |
|---|---|---|
| **8 h average** | | |
| Geometric mean (ppm) | 0.03 | 0.06 |

*Prepared from Hibbs and Gately (1999) and subsequent personal correspondence (SHE Pacific, 2000).

### 5.1.2. Workplace exposure modelling

Workplace exposure (vapour) modelling is a tool routinely used for predicting indoor air concentrations for volatile chemicals. A two-phase steady-state-logarithmic decay model of MOF vapour concentrations in usage rooms was developed; 'Usage Room' is intended to cover use in an ambulance, clinic, aircraft or other enclosed space. The model used an initial steady-state then logarithmic decay conditions according to standard volatilization

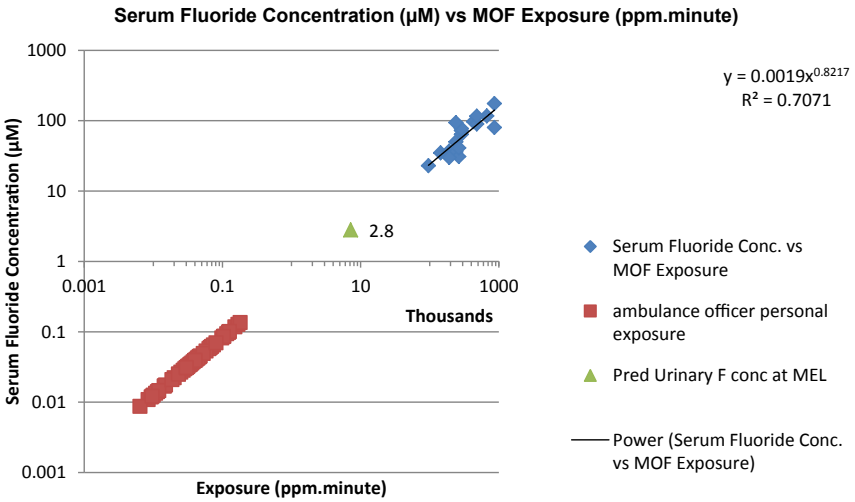



**Fig. 4.** Estimate of Ambulance Officer Fluoride Concentration (red square) and Comparison to Measured Fluoride levels in Cousins and Mazze (1973) (Blue diamond). Using the best fit equation (power $r^2$ of 0.7) of the curve from Cousins and Mazze (1973) the serum fluoride concentration (μM) was estimated from the personal exposure monitoring results for both attendants and drivers (Coffey, 2011). An estimate was also calculated at the MEL.

principles. The steady concentration (first phase) reached by a vapour concentration in an enclosed space is dictated by the rate of evaporation of the compound, the volume of the room, and the ventilation rate. The steady state phase persists until the compound has fully evaporated, at which point vapour concentration is calculated according to exponential decay controlled by the ventilation rate (second phase). The model was based on the following underlying assumptions and calculations:

1. Usage over eight hour shift is assumed at one vial per hour (8 vials) and the worst case is assumed at one vial per half hour (16 vials). Vials are used at regular intervals, i.e. a new vial is dispensed into the inhaler chamber every hour, or every half-hour. This is a highly conservative assumption as it assumes intensive usage. Typical usage would be less than this.
2. The entire contents of the vial (4230 mg MOF) evaporate at a steady state until the inhaler is dry.
3. Dispersal throughout the usage room is immediate with complete mixing, i.e. concentration gradients do not occur.
4. The compound does not react with the atmosphere and is not absorbed by the patient; all methoxyflurane inhaled is exhaled into the atmosphere (i.e. reaction rate, $k$, of zero). In reality, the patient absorbs in the range 65–81% of MOF (Holaday et al., 1970; Yoshimura et al., 1976; Sakai and Takaori, 1978).
5. Empirical data (measurements of MOF leaving the chamber over time) suggest that the minimum evaporation time is 20 min, with an effective usage time of 30 min to 1 h, depending on usage type (continuous or intermittent breathing). Evaporation rate, $E$, is calculated as:

$$E(mg/h) = \frac{mass\ in\ vial}{effective\ usage\ time}$$

Because the minimum usage time gives the highest eight-hour averaged concentration, 0.3333 h (20 min) was assumed:

$$E(mg/h) = \frac{4230\ mg}{0.3333\ h}$$

$$E(mg/h) = 12691\ mg/h$$

6. The steady state concentration ($C_{ss}$; mg/m$^3$) is maintained over the effective usage time, after which the inhaler chamber is dry, and is:

$$C_{ss} = \frac{\frac{E}{V}}{n+k}$$

Where $V$ is the volume of the room, $n$ is the number of air changes per hour, and $k$ is the reaction rate (assumed to be zero as previously described).

7. $C_{ss}$; mg/m$^3$ was converted to ppm, assuming 25 °C, standard pressure, and molecular weight of MOF of 167.94 g/mol:

$$C_{ss}(ppm) = \frac{C_0 x\ 24.45}{molecular\ \ weight}$$

8. Once the inhaler is dry, the concentration of vapour decreases only due to air changes within the room (i.e. does not react with atmosphere and is not absorbed by the patient; reaction rate of zero) according to the relationship:

$$C_t = C_0 e^{-nt}$$

where $C_t$ is the concentration in ppm at time $t$ in hours after release, $C_0$ is the initial concentration after full evaporation of the vial's contents (i.e. $C_{ss}$) and $n$ is the number of air changes per hour.

9. The average concentration over an eight-hour shift (8 h TWA) is the weighted average of concentrations calculated for five minute time steps

$$C_{ave} = \frac{5\Sigma(C_0 + C_{0.083} + \ldots C_8)}{480}$$

10. The peak 1 h TWA is the average vapour concentration over the first hour after opening the vial.

$$C_{ave} = \frac{5\Sigma(C_0 + C_{0.083} + \ldots C_1)}{60}$$

**Table 6**
Input parameters for vapour modelling.

| Parameter | Scenario | TR (treatment room) | Ambulance |
|---|---|---|---|
| ACH (1/h) | Base | 6 | 46 |
| | Min. ventilation | 3 | 26 |
| | Max. ventilation | 15 | 100 |
| Volume ($m^3$) | Base | 32.4 | 11.25 |
| Initial vapour conc. (ppm) | Base | 10 | 4 |
| | Min. ventilation | 19 | 6 |
| | Max. ventilation | 4 | 1.64 |
| Effective usage time (h) | Base | 0.3333 | 0.3333 |

11. For repeated use, the concentration of vapour at any given time step is the sum of the vapour remaining after decay of the most recent vial plus the vapour remaining from previous vials up to 2 h in the past (>2 h the vapour contribution is negligible).

The corollary of these assumptions is that the calculated MOF vapour concentration is an average for the usage room volume overall, not a specific exposure level for a room attendant. Staff in close proximity to a patient using a MOF inhaler may be exposed to a greater concentration than that calculated at least for a short period. However, the model is conservative in its approach in that absorption of MOF by the patient has been discounted to zero, and it assumes the continuous presence of the attendant in the room during the 8 h shift.

The input parameters/variables used in the model were the volume of the usage room, number of air changes per hour (ACH) and number of vials used per day. There is a moderate degree of uncertainty in the ACH assumptions because there are a range of ventilation systems in use, e.g. ducted building ventilation in treatment rooms and fan-driven air conditioning or ventilation in ambulances and aircraft. The ACHs have been interpolated from treatment room design guidelines and observations in US ambulances rather than being taken from direct measurements under Australian conditions. Base, minimum and maximum values used are given in Table 6 Usage rooms were assumed to be an emergency department/hospital, consulting treatment room (TR) and the patient compartment of an ambulance or aircraft. The standard dimension and ventilation of the TR was taken from Australian Health Infrastructure Alliance (AHIA) Australasian Health Facility Guidelines (AusHFG) appendices A and D, and were 12 $m^2 \times 2.7$ m (32.4 $m^3$) and 6 ACH, respectively. These values were used as a base-case in the model. Lower and upper ACH values were taken from US ASHRAE standard 62–2001 *Ventilation for Acceptable Indoor Air Quality* Addendum and HICPAC (2003).

Typically (Munro and Hayes, 2009) an ambulance has a patient compartment volume of 11.25 $m^3$. Ventilation rates observed in US ambulances are 26–46 ACH (Seitz et al., 1996), and therefore, the base-case for the ambulance was 11.25 $m^3$ and 46 ACH, with 26 and 100 ACH taken as lower and upper extremes, representing an approximate two-fold increase and decrease over the baseline condition. This ACH range is supported by measurements in passenger vehicles with the windows closed, the ACH rates of which vary from <2 to >40 depending on the vehicle's velocity and whether the air conditioning and vents were on or off (Ott et al., 2008).

The modelling was intended to reflect ACH variability in ambulance and treatment rooms. The ACH values used for ambulances are also applicable to other modes of transport with comparable ACH such as aircraft.

The final variable tested was effective usage time. Vapour concentration is inversely proportional to the usage time, so minimum (20 min or 0.3333 h) and maximum (1 h) usage times were compared for their effect on 8 h and 1 h TWA in the TR. The shorter exposure at a higher concentration gave higher TWA, so this usage pattern was adopted throughout.

The Penthrox inhaler is fitted with an activated carbon (AC) chamber, which adsorbs MOF exhaled by the patient. Data supplied by the manufacturer (Munro and Hayes, 2009) indicates >86% reduction in fugitive MOF when this is installed. This was included in the model as an AC reduction factor applied to the model outputs.

The model was calibrated against MOF vapour concentrations observed in ambulances (Coffey, 2011). Only ACH and not room volume was varied during calibration of the modelling because initial vapour concentration depends on ACH, ACH values were

**Table 7**
Output vapour concentrations (ppm) from model.

| | | Base[a] | | Min. vent.[b] | | Max. vent.[c] | |
|---|---|---|---|---|---|---|---|
| | | 1 vial/h | 2 vial/h | 1 vial/h | 2 vial/h | 1 vial/h | 2 vial/h |
| TR | 8 h TWA | 0.74 | 1.48 | 1.93 | 3.86 | 0.24 | 0.49 |
| Ambulance | 8 h TWA | 0.21 | 0.43 | 0.39 | 0.77 | 0.1 | 0.2 |

[a] 6 ACH for TR, 46 ACH for ambulance.
[b] 3 ACH for TR, 26 ACH for ambulance.
[c] 15 ACH for TR, 100 ACH for ambulance.

**Table 8**
Comparison of modelled and observed MOF vapour concentrations (ppm) in an ambulance patient compartment.

| (All ppm) | Modelled in patient compartment (1 vial/h) with AC chamber | Observed near attendant without AC chamber |
|---|---|---|
| 8 h TWA – mean | 0.21 (base) | 0.233 |
| 8 h TWA – median | – | 0.150 |
| 8 h TWA – range | 0.1 (min) – 0.39 (max) | 0.048–0.463 (with single outlier 0.048–1.458) |

uncertain while room volumes can be definitively determined, and sensitivity analysis demonstrated that room volume and ACH had similar effects on 8 h TWA. Number of vials was either 1 or 2, at a usage rate of 1 per hour or per half-hour as appropriate, and the scenarios were tested at both usage rates. Outputs of the model under the four scenarios (1 or 2 vials/h, minimum, maximum or base ventilation) are given in Table 6. The AC reduction factor of 85% has been applied to both Ambulance and TR settings as the AC Chamber is typically used in both clinical settings. Ambulance outputs without the AC reduction factor are compared to observations made by Coffey (2011). Outputs are expressed as a function of ventilation (ACH), and are therefore subject to the uncertainty inherent in the ACH rates selected. Reliable estimates of concentrations for specific usage situations will rely on acquisition of ventilation measurements for those situations.

The model estimates are considered reasonable and reliable for estimation purposes. Using the assumed ACH values, modelled outputs (AC chamber is in use) from the base-case ambulance scenario are similar in magnitude to the measured concentrations (where the AC chamber is not in use) in the vicinity of the attendant in ambulance patient compartment (0.21 ppm modelled vs. 0.233 ppm mean and 0.150 ppm median observed) (Tables 7 and 8).

The model demonstrates the importance of ACH on occupational exposure and 8 h TWA. It shows that multiple uses of MOF during a working day has an incremental effect on 8 h TWA. Multiple uses have a smaller effect on 8 h TWA in the ambulance (ACH range modelled 26–100/h) compared to the TR (ACH range 3–15/h). This is because vapour concentrations are dependent on ACH in a logarithmic decay relationship, and decreases rapidly once the MOF in the Inhaler is exhausted (see Figs. 5 and 6 for 1 vial/hour and 2 vial/hour decay curves of ambulance and TR, respectively). It is important that none of the scenarios modelled predicted an 8 h TWA in excess of 15 ppm - maximum predicted 8 h TWA is 3.9 ppm under poor ventilation conditions and 2 vials/h in TR, and 0.8 ppm under the same conditions in the ambulance patient compartment. It should also be noted that this model does not account for MOF absorbed by the patient upon inhalation from the inhaler, which will tend to lead to a further over-estimation of occupational exposure. Approximately 19%–35% of methoxyflurane is exhaled unchanged (Holaday et al., 1970; Yoshimura et al., 1976; Sakai and Takaori, 1978).

Although the focus of the modelling is on 8 h average concentration the modelling also investigated minimum ventilation requirements to keep the peak concentration less than 15 ppm.

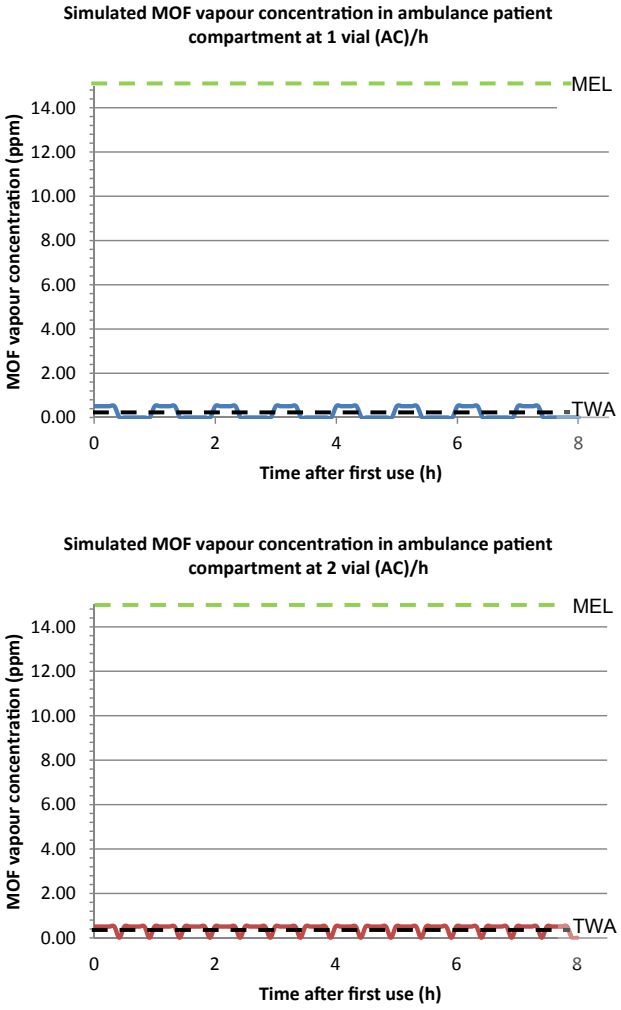



**Fig. 5.** Modelled vapour concentrations in ambulance patient compartment at intensive usage rate of 1 vial/h (top) or 2 vial/h (bottom) with AC Chamber. A volume of 11.25 $m^3$ and ACH of 46/h was assumed. Each peak represents a new vial being dispensed. The TWA (0.21, 0.43 ppm) and peak concentrations remain below the MEL and the concentration of MOF returns to low levels between treatments.

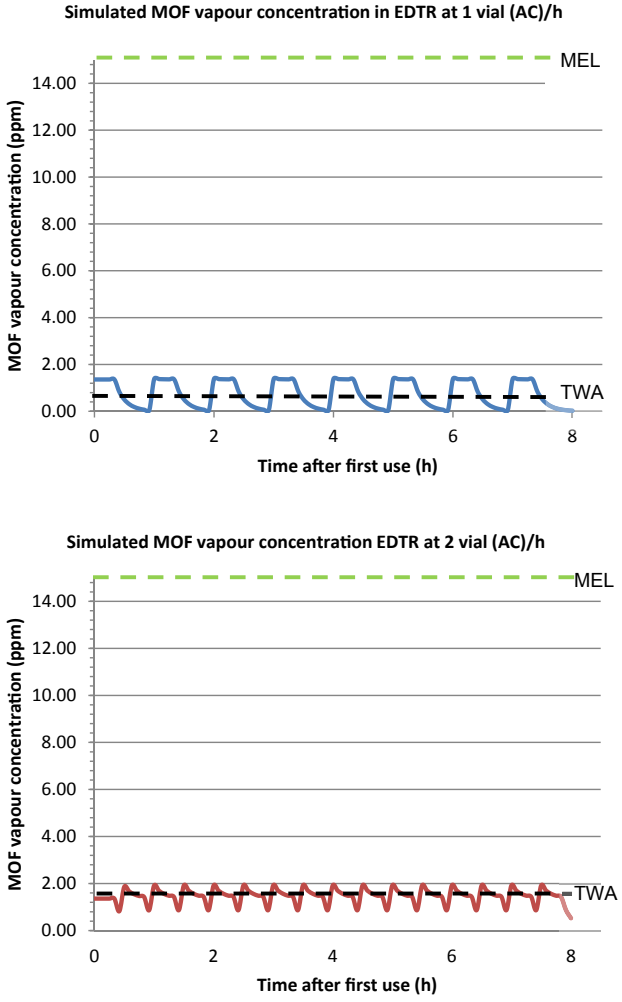



**Fig. 6.** Modelled MOF vapour concentrations in the TR, at intensive usage rates of 1(top) or 2 (bottom) vials/h with AC Chamber, assuming 32.4 $m^3$ volume and 6 ACH. Each peak represents a new vial being dispensed. The TWA (0.74, 1.5 ppm) and peak concentrations remain below the MEL (15 ppm) and the concentration of MOF returns to low levels between treatments.

**Table 9**
Summary of dose response for liver toxicity compared to proposed maximum exposure level.

| Study description | Exposure in ppm min | Effects | Margin[a] |
|---|---|---|---|
| Chenoweth et al., 1972<br>Rats, guinea pigs and rabbits exposed to 200 ppm MOF for 7 h/d, 5 d/wk for 7 wks | 84,000 per day for 7 weeks | Changes in liver function parameters (SGOT, SPGT), focal hepatic infiltration and changes in relative liver weights. | 3 |
| Plummer et al. (1985)<br>Rats exposed to 50 ppm 24 h/d, continuously for 14 weeks | 72,000 *continuously* (24 h/d) for 14 weeks (i.e. this could be interpreted as 6,480,000 ppm min) | Changes in liver weights. Hepatocellular degeneration and necrosis and fatty infiltration. | 2 |
| Egar et al. (1978)<br>Mice exposed to up to 3000 ppm for 2 h/d for last half of pregnancy and 24 exposures at 2–3 day intervals after delivery | 360,000 per day (total of 28 exposures) | No changes in liver weights, no microscopic liver damage observed. | 12 |
| Point of Department (POD) = 30,404 ppm min (based on threshold for nephrotoxicity, refer to Table 4) | | | 1 |

[a] Margin between Liver effect level and point of departure (POD) (i.e. liver effect concentration (ppm min) ÷ POD (ppm min).

The peak is always less than 15 ppm in an ambulance under the following conditions:

1 vial per hour at an ACH of 3; and
2 vial per hour at an ACH of 3.5.

The peak is always less than 15 ppm in a treatment room under the following conditions:

1 vial per hour at an ACH of 1.15; and
2 vial per hour at an ACH of 1.95.

Assuming high intensity use of methoxyflurane the minimum ventilation requirements to keep both the TWA and peak concentrations low are below typical as well as recommended ventilation rates for ambulances (and other vehicles such as aircraft) and treatment rooms demonstrating that the MEL is achieved in worst case simulations of occupational exposure.

The MEL derived is at least 50 times higher than the mean measured observed exposure level for ambulance workers and medical staff involved in supervising the use of Penthrox (refer to Table 8).

Using a model that conservatively estimates occupational exposure assuming high intensity use and typical (base case) ventilation circumstances, the MEL derived is at least 10 times higher than the estimated TWAs.

#### 5.1.3. Odour threshold

The odour detection threshold for MOF was determined using dynamic olfactometry (Munro and Hayes, 2015). This technique involves repeated presentation both of a diluted odour sample and an odour-free air stream to a panel of qualified assessors through two adjacent ports on the olfactometer. Ten trials with six people were conducted in accordance with the methodology described by the Australian Standard AS/NZS 4323.3; 2001.

The odour detection threshold is reported to range between 0.13 and 0.19 ppm (geometric mean 0.15 ppm). Odour could therefore be detected during exposure but the detection threshold was well below the MEL.

### 5.2. Consideration of repeat dose liver toxicity

Severe liver toxicity following MOF exposure during anaesthesia and analgesia has been observed on rare occasions and is considered to be an idiopathic reaction. This form of liver toxicity is postulated to be mediated by the immune system probably involving direct toxicity due to reactive metabolites (Kenna and Jones, 1995). This phenomenon is relatively well understood as it bears similarities to halothane hepatitis, a well described though rare consequence of high and repeat dose exposure.

An underlying presupposition in deriving the MEL is that the critical effect is nephrotoxicity. The biotransformation of MOF results in the release of inorganic fluoride, which inhibits chloride transport in the ascending limb of the loop of Henle (Sweeney and Bromilow, 2006). The release of fluoride is directly related to the duration of exposure, the concentration of MOF and the rate of biotransformation (Sweeney and Bromilow, 2006). Chenoweth et al. (1972) reported non-specific and adaptive changes to the liver in response to prolonged, high level exposure of several species to MOF, quite unlike the clinical and pathological features of halothane hepatitis and as described in the very few cases of hepatotoxicity associated with MOF. Although there are few studies investigating liver toxicity and MOF, the available studies together with the longstanding history of clinical use indicate that protection from kidney effects is very likely also to protect against hepatotoxicity.

Table 9 compares these effect levels to the point of departure chosen for MEL derivation (30,404 ppm min) with the effect levels for liver from repeat dose animal experiments. The POD is between 2 and 12 times lower than the effect levels for liver toxicity supporting the selection of kidney as the POD.

## 6. Conclusions

The analysis in this paper highlights the value of a combination of the breadth and depth of a dataset obtained for clinical evaluation with modern risk assessment techniques used to derive occupational exposure levels. The toxicity database of methoxyflurane is relatively unique given its history of use in anaesthesia (high acute dose) and exposure to lower intermittent dosage as an analgesic. Translating this dataset to an intermittent occupational exposure (low dose) is demonstrated within the paper. The interpretation and extrapolation of clinical toxicity data for occupational risk assessment is detailed taking account of the change in pattern of the exposure from a high concentration single exposure to a low concentration intermittent occupational exposure. A maximum exposure limit (MEL) of 15 ppm as a time weighted average has been derived with a degree of conservatism to match the uncertainties inherent in the toxicity dataset. The MEL of 15 ppm is a conservative benchmark that should allow a considerable margin of protection of occupational health during the responsible use of Penthrox.

Similarly conservative assumptions have been employed in a modelling exercise to explore possible ambient exposures of staff supervising the self-administration of MOF in a variety of settings related to likely working practices in ambulances, treatment rooms and aircraft. The predicted exposures were checked against real-world measurements and both indicated a significant margin of

safety of between 10 and 50 even when very conservative assumptions were made about the possible use of MOF.

## Conflict of interest

The manuscript authors have no conflicts to disclose.

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## Transparency document

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