

Letters to the Editor

Tribromoethanol (Avertin) as an anaesthetic in mice

Zeller *et al.* (1998) have described the 'adverse effects of tribromoethanol as used in the production of transgenic mice'. The authors reported focal to diffuse necrosis of the abdominal wall and necrotic changes on the surfaces of the abdominal organs and concluded that the use of tribromoethanol is not to be recommended as a mouse anaesthetic agent. For several reasons we disagree with their conclusion.

Tribromoethanol has been successfully used as an anaesthetic in mice by numerous transgene laboratories throughout the world for almost 15 years (Hogan *et al.* 1986, Monk 1987, Robertson 1987, Joyner 1993, Murphy & Carter 1993, Pinkert 1994, Hogan *et al.* 1996). Reasons cited for its popularity are that tribromoethanol provides rapid and deep anaesthesia in mice, followed by fast and full postoperative recovery. Moreover, additional dosing that may be required is well-tolerated by the animals, without any noticeable adverse postoperative outcome. Surgical anaesthesia (negative pedal reflex) is achieved within 5 min of i.p. injection of the drug, and anaesthesia is maintained for 20–30 min, which is sufficient for performing bilateral embryo transfers. Finally, the animals are fully mobile within approximately 80 min and are often eating or drinking again after 100 min.

By contrast, mice administered ketamine/xylazine (0.1 ml/10 g body weight) remain anaesthetized for 30–40 min, followed by a 5–10 min phase of shivering after awakening. These animals often exhibit a more or less dramatic bristled coat for about 30 min, which may be from loss of temperature and/or a sign of general distress.

In our laboratory during the past 10 years more than 5000 mice have been anaesthetized with tribromoethanol, using the methods of Hogan *et al.* (1986, 1994), for embryo transfers, vasectomies and other surgical protocols. The anaesthetic is freshly prepared from the stock solution according to Hogan *et al.* (1996) and sterile filtrated before use for each experimental day. The typical dose is 20 ml tribromoethanol (1.25%)/g body weight injected i.p. in younger mice (NMRI: until 16 weeks) and 30 ml drug/g body weight in older individuals.

Only one animal of these thousands of treated mice died as a result of anaesthetic administration, and necropsy revealed that this was because of an improper injection, which led to the rupture of the abdominal aorta. There were no intraoperative nor postoperative incidents in any of the other animals. At least 250 of the mice underwent necropsies, and no visceral adhesions or lesions, or signs of infections were detected. In contrast, foster mothers (NMRI) remained healthy and gave birth to large

litters with a considerable percentage of founders (average >6 founders per experiment). Vasectomized males served for 1–1.5 years and showed high rates of species-typical mating behaviours.

Clearly, our experiences with tribromoethanol are not consonant with the results reported by Zeller *et al.* (1998). We suspect that one source of these starkly different experiences of Zeller *et al.* (1998) may be due to the different methods of preparing and handling tribromoethanol for anaesthesia.

- (1) Zeller *et al.* (1998) describe only dissolving the (powdery) tribromoethanol in Hank's balanced salts, instead of using tertiary amyl alcohol as a diluent, as recommended by Hogan *et al.* (1986). The polarity of Hank's is too high to allow tribromoethanol to be solubilized at room temperature, leaving a significant quantity of undissolved crystals within the anaesthetic solution.
- (2) Mice are susceptible to infections, as are other species, if microorganisms are transported into their peritoneum. It is therefore strongly recommended to perform sterile filtration of the anaesthetic solution before it is administered to the animal. While common anaesthetics such as ketamine/xylazine are sold as sterile solutions and ready for use, neither tribromoethanol nor Hank's balanced salts have that feature. Moreover, the solubilization process poses additional risks of microbial contamination. Zeller *et al.* (1998) did not mention using sterile filtration or other methods to reduce contamination risks.
- (3) Tribromoethanol molecules are highly light sensitive and will respond to light exposure by rapid degradation. Both stock solutions and the solution ready for use should therefore be stored thoroughly wrapped in foil. Zeller *et al.* (1998) did not report storage methods, raising the possibility that the drug underwent degradation prior to administration.

To summarize, our experiences, and the experiences of numerous other transgene laboratories, recommend tribromoethanol as a quite suitable anaesthetic for mice. When the drug is prepared and handled properly, there appears to be little risk of the adverse consequences described by Zeller *et al.* (1998).

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The author's reply

We would like to thank Drs Weiss and Zimmermann for their comments, and reply to the issues raised as follows:

- (1) As described in 'materials and methods' of our paper, we dissolved tribromoethanol in tertiary amyl alcohol (group 4, concentration of tribromoethanol 1.2%). In this group histopathological changes were evident.
- (2) The solvents used were sterile, but we did not perform sterile filtration of the finished solution. Nevertheless, there were no clinical signs of bacterial peritonitis in mice anaesthetized with tribromoethanol. The absence of clinical signs applies both for the experiments reported in our paper and for all animals following routine embryo transfer.

In a recent follow-up study with blinded histopathological examination, we used only freshly prepared and sterile-filtered tribromoethanol. Depending on the concentration of tribromoethanol, we again found serositis and necrosis although, in general, this was less

- pronounced than previously. It may indeed be possible that sterile filtration of the solution reduces the quantity of undissolved crystals. This reduction could result in a diminished irritant effect of the tribromoethanol solution.
- (3) Only freshly prepared tribromoethanol solutions were used in our experiments.

It is well known that a decrease in body temperature accompanies any kind of general anaesthesia, which may be counteracted by employing an external heat source during and after surgery. Shivering during or following the recovery phase indicates a body temperature below normal. In our experience the drop in body temperature is more marked following anaesthesia with tribromoethanol than with ketamine/xylazine, and a greater distance between warming lamp and animals is necessary to prevent overheating when using ketamine/xylazine anaesthesia.

In summary we conclude that bacterial infection is unlikely to be the cause of the lesions observed. Tribromoethanol itself possesses a concentration-dependent irritant effect, and further, decomposition of dissolved tribromoethanol to toxic components may result from improper storage. However it is not normal practice to prepare tribromoethanol in the appropriate manner, followed by sterile filtration, on a daily basis.

No such disadvantages are reported using ketamine/xylazine for anaesthesia. In addition, there is no relevant difference between the two anaesthetic methods regarding the successful outcome of the embryo transfer. We therefore recommend that the use of tribromoethanol be discontinued.

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