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Letter to the Editor

Ethics and Animal Welfare Related to *in vivo* Pharmacology and Toxicology in Laboratory Animals

Over the last decade there has been an increasing focus on ethics, animal welfare and humane end-points in laboratory animal units, laboratories, different learned societies and editorial boards of international scientific journals publishing work from *in vivo* pharmacological or *in vivo* toxicological studies in laboratory animals. The European society and maybe particularly the Northern European societies have had a strong focus on these essential topics. This has had an impact on the Council of Europe, who recently revised the Appendix A to *The European Convention for protection of vertebrate animals used for experimental and other scientific purposes (1986, ETS 123)* on a number of topics with ethics and animal welfare as the driving force. For certain animal species, the Convention is still under revision. The Convention has basically been adopted into EU legislation and some of the recent revisions of Appendix A have been introduced into national legislation of some EU member countries. Based on this somewhat complicated situation, licensing authorities may in some EU countries prohibit licensing for certain procedures in laboratory animals, whereas exactly the same procedure may be accepted for licensing in another country.

For international scientific journals publishing *in vivo* pharmacological or toxicological studies, this variation in ethics and its impact on animal welfare may pose difficulties to editors and referees, because manuscripts stating that the study was approved from an animal welfare point by national committees in the country where the work was carried out, may not have been approved in the country of the individual referee, the editor or the editorial board.

In the May 2005 issue of *Basic & Clinical Pharmacology & Toxicology*, two publications raise concern about appropriate animal welfare, protection of animals and humane end-points.

In the paper by Muriel *et al.* (2005), death was used as an end-point to describe the tolerability of treatment with CCl_4 as a tool to induce liver fibrosis. In total 76 out of 144 rats died.

In addition, a dose of 400 mg/kg of CCl_4 dissolved in mineral oil was administered intraperitoneally three times a week for up to sixteen weeks, in total 48 intraperitoneal injections. The concern about intraperitoneal injections is dealt with in detail below.

Death is an ultimate end-point and often includes severe suffering before death and in particular in the late period. Death can be accepted as an end-point under certain conditions. There are still regulatory requirements according to which death should be used as an end-point, in particular in the quality control testing of new batches of vaccines but also for other purposes. In some studies, for instance with anaesthetics, death can be accepted as an end-point because suffering is prevented by the fact that the animals are unconscious. However, in many situations considerably more humane end-points can be used in order to describe an effect, which eventually might lead to death, at a much earlier stage in the process. These may be

clinical symptoms including body weight developed to a stage of no return, where the animals are then killed. Alternatively, end-points as certain clinical pathology parameters may be used as indicators of a stage of no return, after which the animals are killed. There are many other examples, and essentially it is a matter of creativity when study protocols are designed.

In the paper by Luger *et al.* (2005) the formulations of antibiotics were administered intraperitoneally. The dose volume was 2 ml, which was administered over 20 sec. The dose volume contained the desired dose of the antibiotic. The dose of gentamicin ranged from 0.1–10 mg/kg and that of ciprofloxacin from 1–100 mg/kg. Consequently, the concentrations of the formulations have for gentamicin ranged from 0.0125–1.25 mg/ml for a 250 g rat and for ciprofloxacin from 0.125–12.5 mg/ml. In parts of the study naloxone was administered intraperitoneally 30 min. after intraperitoneal injection of gentamicin or ciprofloxacin. In these cases two intraperitoneal injections were given. Basically, intraperitoneal injections are injections into a black box and for this reason the procedure causes a number of concerns. The injection is very easy to perform compared to many other routes of parenteral administration. However, with intraperitoneal injection there are absorption, tolerance and misplacement issues to be taken into consideration. Absorption can vary considerably with respect to visceral absorption depending on where the injection is placed (Claassen 1994). In case of visceral absorption the drug absorbed will subsequently enter the portal vein and may be subject to first-pass metabolism in the liver. The visceral absorption is different in different parts of the abdominal cavity. At the diaphragm level there is also substantial lymphogenic absorption. Drug formulations might cause irritation to the parietal and visceral peritoneum and the underlying tissues. This is well known for formulations of barbiturates or with acetic acid, and many other formulations. The irritation may affect the absorption rate and in addition cause peritoneal and visceral pain. Further, intraperitoneal injections of different formulations have been used as a tool to induce peritoneal sclerosis in mice and rats (Gotloib *et al.* 2005). Misplacement of the formulation injected is highly likely for some of the animals and thus another confounding factor. At *post mortem* examination many examples of intestinal injection have been seen either in or into the small and large intestines depending on animal species. Misplacements in the liver, spleen and seminal vesicle are frequent. In a number of papers it has been reported that error of placement of formulations after intraperitoneal injection in mice or rats varies from 10 to 24% (Lewis *et al.* 1966; Steward *et al.* 1968; Miner *et al.* 1969; Arioli & Rossi 1970; Schneider & Scheider 1970; Walvoort 1991; Claassen 1994). However with a two-person procedure the incidence of error was substantially reduced in the study by Arioli & Rossi (1970), but in other studies optimization of the procedure did not reduce the incidence of errors (Miner *et al.* 1969; Walvoort 1991). All these factors add to the variation in pharmacokinetic and pharmacodynamic endpoints to be measured. In addition, the procedure has been shown to be potentially stressful to the animals as indicated by expression of Fos antigens in discrete areas of the brain of mice after intraperitoneal injection of normal saline (Ryabinin *et al.* 1999) or by elevation of nociceptive threshold after intraperitoneal injection of hypertonic saline as stress-induced analgesia (Wright & Lincoln 1985).

Absorption from subcutaneous injection is not affected to the same degree by confounding factors. In addition, subcutaneous injections are almost similarly easy to perform. For this reason subcutaneous injection is a favourable alternative to intraperitoneal injection, and the study might need fewer animals because the variation is less. There may be situations where intraperitoneal injection has priority but in those situations it should ideally be justified.

In the study by Muriel *et al*. (2005) mineral oil was used as vehicle for CCl_4. It is well known that mineral oils cause granulomateous inflammation at the site of injection, whereas this is not the case for vegetable oils. The paper does not describe granulomateous reactions in the abdominal cavity or abdominal organs, probably because this was not the aim of the study.

There are many publications on ethics, animal welfare and humane end-points in the scientific literature. Some of these publications which focus particularly on pharmacology and toxicology, are given in the reference list below (Sandø & Svendsen 1997; Svendsen *et al.* 1997; Hansen *et all.* 1999). The messages given may be useful for editorial board members and referees of this journal, when considering acceptance of manuscripts involving experimental work with laboratory animals. This is further substantiated by the workshop publication "Humane endpoints in animal experiments for biomedical research" (Hendriksen & Morton 1999) where research areas involving laboratory animals causing major concern were listed. Major concern was related to medical research in cancer, transplantation, drug development (pharmacology) and toxicology, so pharmacology and toxicology are the disciplines where animal welfare issues should be in focus.

Ove Svendsen
Department of Veterinary Pathobiology
The Royal Veterinary and Agricultural University
DK-1870 Frederiksberg
Denmark

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