THE WELFARE IMPACT OF INCREASED GAVAGING DOSES IN RATS

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Abstract *Animal Welfare 2001,* ***10:*** 303-314

Textbook recommendations for gavaging rats vary between 1–5 ml for an adult rat. Rats weighing either 130 g or 250 g were gavaged with varying dosages of barium sulphate ($BaSO_4$). After dosing, radiographs were taken at 0, 15 and 60 min. Animals showing a section of the small intestine totally filled with $BaSO_4$ were scored as displaying spontaneous release. Other rats of the same sizes were gavaged with similar doses and subsequently tested in an open-field arena for behavioural abnormalities that might indicate stress or pain resulting from the procedure. Body temperature before and after treatment was recorded using microchip transponders. None of the 250 g rats in the 1 ml dosage group showed spontaneous release through the pyloric sphincter. In the 2 ml and 4 ml dosage groups, only one out of five animals showed spontaneous release. In the 6 ml dosage group, half of the animals showed spontaneous release. In the 8 ml and 10 ml dosage groups, five out of six and four out of five, respectively, showed spontaneous release. If doses were higher than 12 ml, no animal was able to keep all of the $BaSO_4$ in its stomach. In the rats weighing 130 g, the 3 ml dosage group showed only one out of four rats with spontaneous release, whereas in the 5 ml and 7 ml dosage groups, all animals showed spontaneous release. After 15 min, all of the rats in both weight groups showed $BaSO_4$ in the duodenum. Ambulation, rearing up onto the hind legs and defecation, as well as body temperature immediately after dosing, correlated very strongly with the dose (ml kg^{-1}); increasing the dose resulted in reduced ambulation, rearing, defecation and body temperature. However, 10 min after performance of the open-field test, neither body temperature, serum corticosterone nor serum glucose showed any correlation with dose. This study indicates that high doses (ie doses up to 10 ml for a 250 g rat) might be safe to use; however, if an adverse impact on the rat is to be avoided, use of much lower doses should be considered — for example, doses that do not enforce opening of the pyloric sphincter in any rat. This would be less than 4 ml kg^{-1} in a 250 g rat.

Keywords: *animal welfare, body temperature, corticosterone, gavage, open-field test, radiocontrast, rat, sphincter pylori, welfare*

Introduction

Oral administration of drugs or other substances through a tube, known as gavaging, is a frequently used method in research involving laboratory rats. Different recommendations of acceptable maximum volumes may be found in various textbooks of laboratory animal science ranging between 1–5 ml for an adult rat (Iwarsson *et al* 1994; Fallon 1996; Hillyer & Quesenberry 1997). Such recommendations seem to be based upon best practice rather than scientific empirical evidence, and it is unclear whether the impact on the welfare of the animal or the outcome of the study has been the main consideration in the recommendations. Higher doses are often used. Certain types of viscous oils may induce a corticosterone response in the rat, whereas less viscous substances may induce a higher risk of aspiration of the compound into the lungs. On the basis of our current knowledge, a maximum dose of 20 ml kg^{-1} body weight has been proposed (Brown *et al* 2000) but, in fact, fairly little is known about the impact of increased doses on the rat.

It is reasonable to believe that high doses may make an animal may feel uncomfortable, but if part of the dose passes into the duodenum the discomfort may be less than if the compound is retained in the stomach. The location of the compound can be examined by dosing rats with a radiocontrast agent such as barium sulphate ($BaSO_4$). It is a far more complex matter to monitor the full impact that the dose has on the animal. Gavaging of a rat may result in a 'feeling' that may be described by the physiological term 'pain', although in a more clinical sense the feeling may be graded from light nausea to discomfort to strong pain. Pain impulses are passed from local nociceptors to the cerebral cortex, where they are translated into conscious feelings. Pain may work as a stressor that the animal cannot easily cope with, and this may lead to a hypothalamic–pituitary response — in other words, physiological stress. Traditionally, such stress has been thought to result from the pituitary release of adrenocorticotrophic hormone and the subsequent release of corticoids from the adrenal cortex, but several pituitary hormones are involved (Armario *et al* 1986; Broom & Johnson 1993). Stress is related to fear but is distinct: fear is more closely related to the adrenal medulla and the sympathetic nervous system. A stressed animal will behave differently from a control rat (Bateson 1991) and, therefore, behavioural analysis is a simple yet effective way of assessing welfare (Baumans *et al* 1994). The open-field arena (Göb *et al* 1987), in combination with remote control videotaping (Liles *et al* 1998), is a frequently used tool for evaluation of animal behaviour. In the open-field arena, rats are known to display exploratory behaviour. Three behavioural elements of the open-field test that have been shown to be reliable measures when assessing rat welfare are: first, ambulation (measured by the number of segments of the field crossed); second, rearing up onto the hind legs; and third, defecation (Ivinskis 1968). An unstressed rat immediately starts exploring, moving about, rearing up on the hind legs to obtain a better view, and looking for potential ways to escape, food, conspecifics, predators, etc. (Barnett 1975). Abnormal behaviour is defined as any deviation from this, and such an abnormality might suggest stress in the individual tested. Defecation my change in either direction in a stressed rat. With this knowledge, it is possible to detect deviations from species- and strain-dependent rat behaviour displayed in the open field, although the deviations shown may be difficult to interpret because of their complex background. Differences in exploration related to the rat's social status have been described by Williams and Lierle (1988).

Body temperature may serve as an accurate indicator of stress (Bateson 1991; Georgiev 1978; Kort *et al* 1998; Long *et al* 1991). An acute reaction to immobilisation, for example, may be shown as a drop in body temperature, which is probably dopamine-related (Amar &

Sanyal 1981); this is followed after about one hour by an increase in body temperature, which is probably related to prostaglandins (Singer *et al* 1986). Any reaction to gavaging must, therefore, be monitored both immediately after the procedure and again after the open-field trial. As an immediate response to stress, a temperature drop should be expected. This reaction is attributable to the immobilisation during the procedure. The activities in the open-field arena themselves lead to a prostaglandin-related rise in body temperature (Singer *et al* 1986), but an animal additionally stressed by the gavaging procedure may have a higher increase than control animals. The impact of the immobilisation itself may be minimised by gentling, a procedure in which the researcher slowly habituates the animal to human contact and handling by daily gentle petting of each individual followed by the carrying of the animal. Gentling has been shown to reduce fear of handling procedures (Hirsjärvi & Väliaho 1995; Seggie & Brown 1974).

In order to evaluate the welfare impact of increasing gavaging doses so that recommendations for dose limitations can be made, it is essential to study both the fate of the gavaged dose as well as the impact that the dose has on the animal. The present study was designed to investigate the influence of gavaging on the welfare of rats; it should be kept in mind that reduced animal welfare may also reduce the scientific quality of the experiment (Barnett 1975; Broom & Johnson 1993; Howard 1997).

Materials and methods

Animals and husbandry

All rats used were male barrier-raised Pan:Wistar rats health-monitored in accordance with FELASA guidelines (Kraft *et al* 1994) testing positive only to *Pasteurella pneumotropica*. The animals were kept in groups of two at 22 ± 1 °C at a relative humidity of 50–70 per cent, under a dark–light schedule of 12:12 hrs (lights on from 0600h to 1800h) in transparent Macrolon type-three cages (Scanbur Ltd, Lellinge, Denmark) on aspen bedding (Finn Tapvei, Kaavi, Finland), and fed Altromin 1314 diet (Altromin, Gentofte, Denmark).

Gavaging

Syringes of 5, 10 and 20 ml capacity and probe-ended stainless-steel gastric tubes 10 cm long with an external diameter of 2 mm were used for gavaging $BaSO_4$ (1 g ml^{-1}, Micropaque, Laboratoires Roche-Nicholas, France). Prior to the administration of $BaSO_4$, the rats had been fasting for 24 h with free access to water.

Experimental design for the radiographic measurement

Forty-two rats weighing around 250 g and 12 rats weighing around 130 g were used. Animals weighing 250 g were divided into eight groups of five animals. Two surplus animals were used as extra animals in the 6 ml and 8 ml groups, which then contained six animals. After arrival at the animal unit, all animals were allowed one week of acclimatisation. The individuals in each group were dosed with one of the following doses: 1, 2, 4, 6, 8, 10, 12, or 14 ml of $BaSO_4$ heated to 38 °C. The 130 g rats were divided into three groups of four animals and were dosed with either 3, 5 or 7 ml of $BaSO_4$ heated to 38 °C. A 10 cm long metal gavage needle with an external diameter of 2 mm (B&K Universal, Albertslund, Denmark) was used for gavaging. Immediately after dosing, a radiograph of each rat was taken, and this was repeated at 15 and 60 minutes. For the radiographs, 0.10 s, 20 mA and 70 kV were used. All animals were immobilised in a plexiglass trap (half cylinder type, 18 x 7 cm) with no further restraint for the radiograph, and immediately afterwards they were returned to their home cages (Svendsen & Hansen 1999). Any animal showing a section of the small intestine filled

with $BaSO_4$ was scored as 'release'. Animals showing no, or only faint, traces of the contrast in the small intestine were scored as 'no release'.

Experimental design for studies of the impact on behaviour and physiology

Forty-one rats were used. After arrival at the animal unit, all animals were allowed one week of acclimatisation during which each individual was gentled for five minutes a day. On the second day of acclimatisation, all animals were injected subcutaneously with 0.3 ml lidocaine (20 mg ml^{-1}; Pharmacy of the Royal Veterinary and Agricultural University, Copenhagen), and a microtransponder for measuring body temperature and identification code (BMDS IPTT Implantable Programmable Temperature Transponder, PLEXX, Elst, Netherlands) was implanted subcutaneously in the neck region. Daily scanning (DAS-5007 IPTT Pocket Scanner, PLEXX, Elst, Netherlands) of the transponders was performed during the remaining acclimatisation period to adjust animals to this procedure.

Treatment and behavioural tests were carried out in the period between 0800h and 1200h in two rooms separated from the animal unit. For the open-field test, an arena made of aluminium measuring 100 cm long x 150 cm wide x 40 cm high was used. The floor was covered with bedding (Spanwall White Special, Hørve, Denmark). Lines (squares of 10 x 10 cm) were drawn on the TV screen, with the central field of the arena measuring 50 x 40 cm surrounding the novelty object (cardboard box with identification number written on top). The video equipment was placed in a separate room.

Twenty-four of the rats each weighed around 280 g. Eight of these were used as the control group, and the other sixteen were divided into four treatment groups, each comprising four rats. One rat in the 12 ml group could not easily be gavaged and was, therefore, not used for further experiments. Seventeen rats weighing around 130 g were also used. Eight of these were used as the control group, and nine were divided into three treatment groups, each comprising three rats. One rat in the 5 ml group could not easily be gavaged and was, therefore, not used for further experiments. The 280 g rats of each group were dosed with one of the following doses: 0, 8, 10, 12, or 14 ml of $BaSO_4$, while the 130 g rats were dosed with one of the following doses: 0, 3, 5, or 7 ml of $BaSO_4$. Control animals were treated identically to the test animals, except for the fact that nothing was dosed through the tube. All animals were randomly assigned to either the control or the treatment groups.

Before treatment, animals were scanned in their homecage for identification and measurement of body temperature. Treatment with one of the above-mentioned doses was followed by a subsequent temperature scanning and within one minute the animal was introduced into the open-field arena.

Behaviour was recorded using TV video equipment so that the animals were not affected by the experimenter's sounds and possible disturbing odours (Camera: CCD with CCTV Lens 1/3", Philips; VCR: AG-7330, Panasonic; TV: JVC TM-20PSN). During the ten minutes of observation, neither other animals nor people were present in the room. Faecal pellets and the bedding surrounding these pellets were removed after each rat, and between every six animals all bedding was changed and the open-field arena washed in hot water. The videotape was studied and behaviour was recorded for each rat.

After observation, a third scanning was performed and the rat was subsequently removed from the open field and anaesthetised with fentanyl/fluanison/midazolam (Hypnorm™, Janssen-Cilag, Birkerød, Denmark/Dormicum™, Roche, Basel, Switzerland) as described by Iwarsson (1994). A blood sample was taken retro-orbitally to monitor concentrations of plasma corticosterone (Coat-A-Count Rat Corticosterone assay, Diagnostic Products

Corporation, Los Angeles, CA, USA) and plasma glucose (Refloton analyzer and Refloton Glucose sticks, Boehringer Mannheim, Mannheim, Germany).

After recovery, all animals were placed in their own cage with their original cage-mate overnight. The next day, all animals were tested to establish their rank using an intruder test (Hart 1985), in which an unknown conspecific is placed in the homecage and the first attack from one of the resident rats (the dominant rat) is noted. Thereafter, all rats were killed humanely with a 20 per cent solution of pentobarbital injected intraperitoneally.

Statistics

For each weight group, correlation coefficients were calculated to describe the association between the dose and the number of animals with spontaneous release and the behavioural and physiological parameters. Next, the calculated correlation coefficients were tested using analysis of variance (ANOVA) for difference from zero. All tests were carried out using the software Minitab, Release 12.1 (Minitab Inc., State College, Philadelphia, USA). To compensate for the high degree of individual variation normally found when monitoring body temperature of rats using these microchips, body temperatures used for evaluation were calculated as the ratio between the individual's temperature after treatment/open-field test and its temperature before treatment.

Results

Radiographic examinations

Figure 1 shows radiographs of 250 g rats immediately after dosing with 1 ml, 6 ml and 14 ml $BaSO_4$, illustrating both full retention of the dose and some of the dose emptying into the duodenum.

The fraction of animals showing spontaneous release in relation to dosage is shown in Table 1 and Figure 2. Among the 250 g rats, there was a positive correlation between dosage and number of animals showing spontaneous release ($P < 0.001$). All of the 250 g rats showed release after 15 min. Those animals dosed with volumes of 12 ml or more showed some discomfort during dosing, including cyanosis at the end of the gavaging phase. The number of 130 g rats showing spontaneous release is also shown in Table 1. Only one out of four rats dosed with 3 ml showed spontaneous release, whereas in the dosage groups of 5 ml and 7 ml, all animals showed spontaneous release. All the animals of this weight group showed release after 15 min. The rats in the 7 ml group seemed to oppose gavaging more than the other dosage groups. No linear correlation between the number of animals showing spontaneous release and the dose could be demonstrated.

Impact on behaviour

The behavioural observations are shown in Figures 3, 4 and 5. For all rats, when the results were pooled, ambulation ($P < 0.001$), rearing ($P < 0.004$) and defecation ($P < 0.013$) were significantly reduced by increasing doses. This was similar when studying both 130 g rats and 280 g rats independently of one another, except for the fact that significance could not be shown for rearing in 130 g rats and defecation in 280 g rats. No differences relating to the order in which the animals were tested were found; neither were any differences in relation to social status found using the intruder test.

Table 1 **The fraction of rats showing spontaneous release from the stomach into the duodenum when gavaged with different volumes of $BaSO_4$.**

250 g rats								
$BaSO_4$ volume (ml)	1	2	4	6	8	10	12	14
$BaSO_4$ volume (ml/kg)	4	8	16	24	32	40	48	56
No. of animals with release/total number of dosed animals								
Immediate	0/5	1/5	1/5	3/6	5/6	4/5	5/5	5/5
15 minutes	5/5	5/5	5/5	6/6	6/6	5/5	5/5	5/5
60 minutes	5/5	5/5	5/5	6/6	6/6	5/5	5/5	5/5
130 g rats								
$BaSO_4$ volume (ml)			3		5		7	
$BaSO_4$ volume (ml/kg)			23		38		54	
No. of animals with release/total number of dosed animals								
Immediate			1/4		4/4		4/4	
15 minutes			4/4		4/4		4/4	
60 minutes			4/4		4/4		4/4	

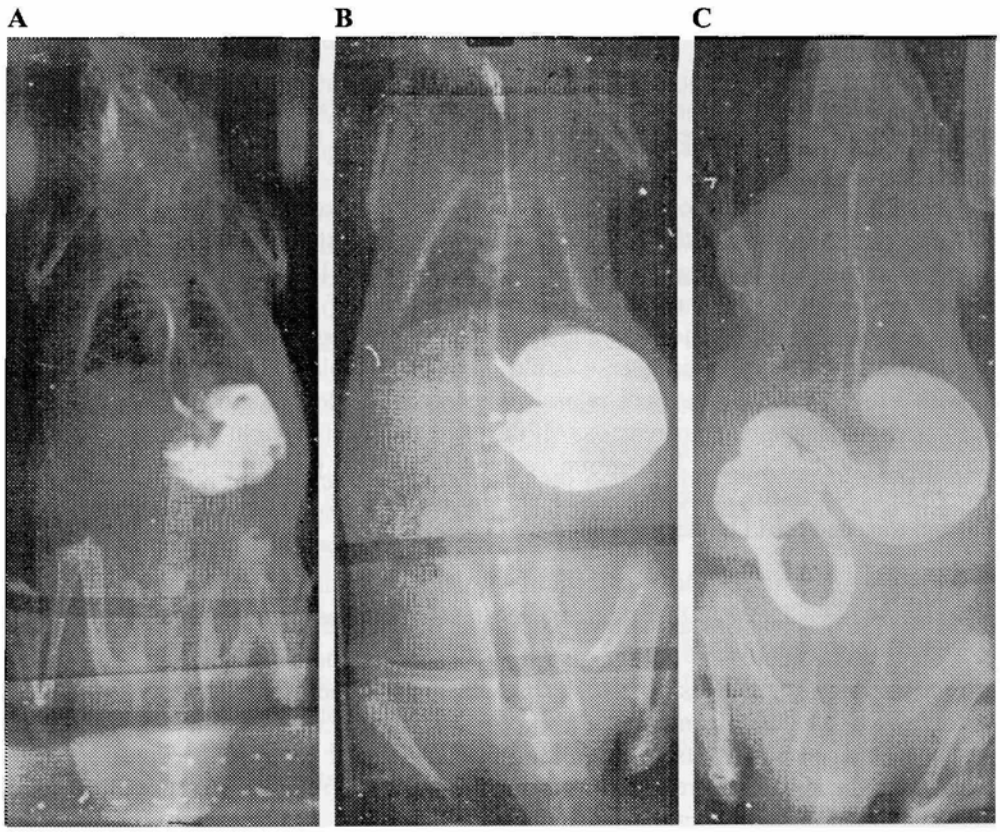

Figure 1 **Radiographs of 250 g rats after dosing with (A) 1 ml $BaSO_4$, (B) 6 ml $BaSO_4$, and (C) 14 ml $BaSO_4$. In (A) and (B), the entire dosage has been retained within the stomach, whereas in (C), the dosage has immediately been released into the duodenum.**

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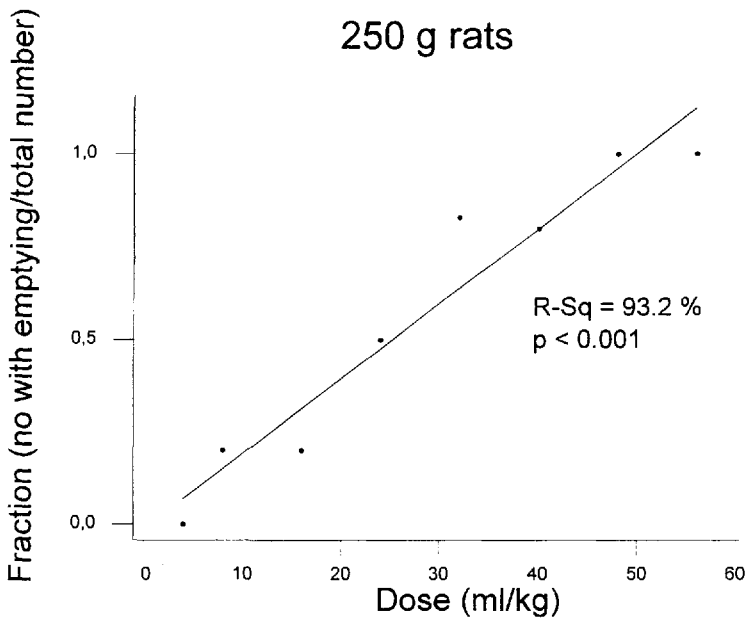

Figure 2 **The relation between the number of rats showing spontaneous release from the stomach into the duodenum and the dose with which they were gavaged.**

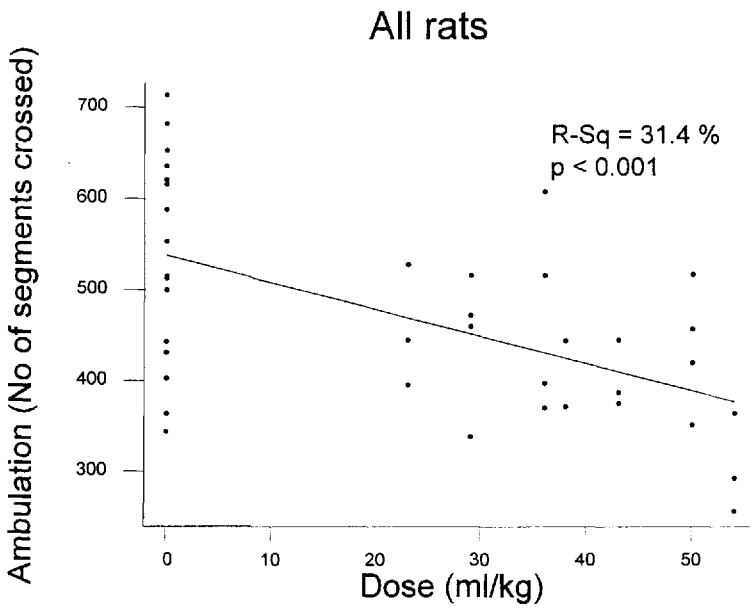

Figure 3 **The relation between ambulation of Pan:WIST rats in the open field and gavaging with various volumes of barium sulphate. Rats of two weight groups were tested and a significant correlation was found for both 130 g rats (R-Sq = 48.2%, $P < 0.05$) and 280 g rats (R-Sq = 24.2%, $P < 0.05$).**

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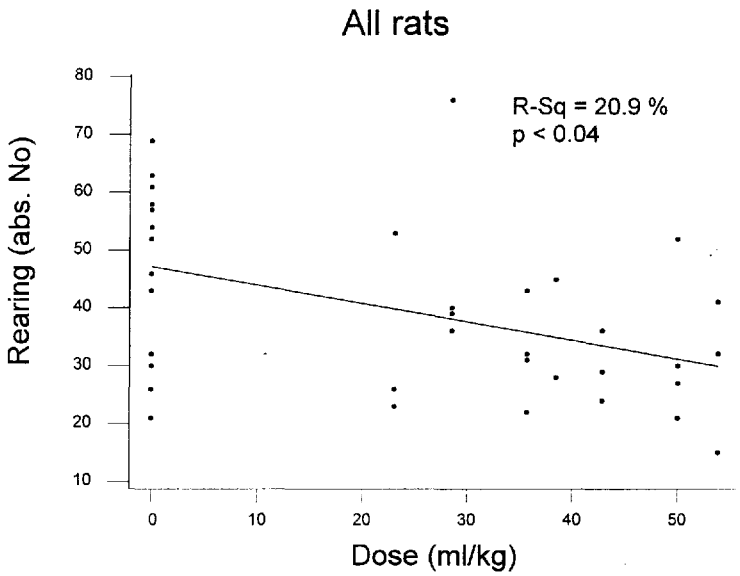

Figure 4 **The relation between rearing of Pan:WIST rats in the open field and gavaging with various volumes of barium sulphate. Rats of two weight groups were tested. A significant correlation was found for 280 g rats (R-Sq = 36.0%, $P < 0.03$), whereas no significant correlation was found for 130 g rats (R-Sq = 11.4%, $P = 0.201$), when tested independently.**

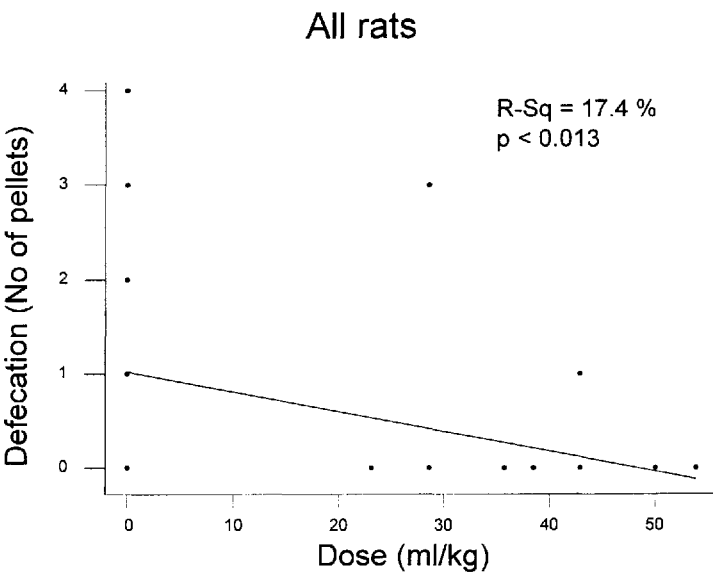

Figure 5 **The relation between defecation of Pan:WIST rats in the open field and gavaging with various volumes of barium sulphate. Rats of two weight groups were tested. A significant correlation was found for 130 g rats (R-Sq = 33.0%, $P < 0.05$), whereas no significant correlation was found for 280 g rats (R-Sq = 13.6%, $P = 0.084$), when tested independently.**

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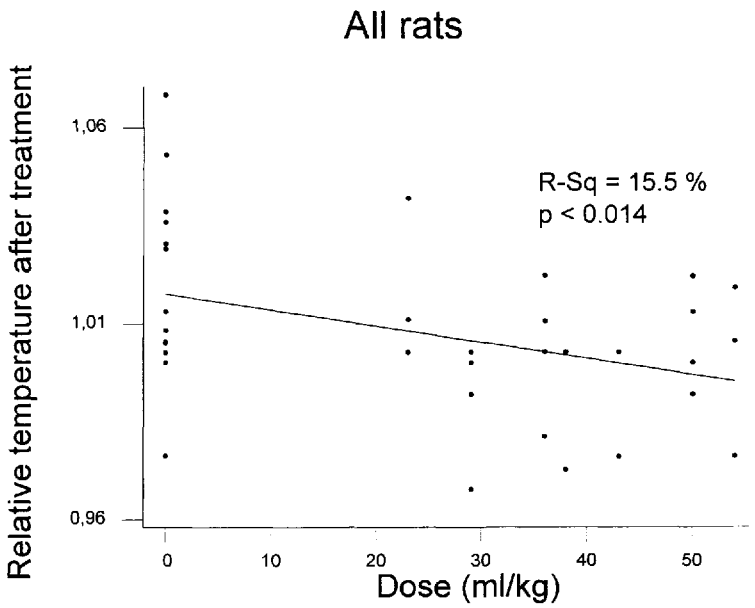

Figure 6 **The relation between relative body temperatures in Pan:WIST rats and gavaging with various volumes of barium sulphate as monitored immediately after the procedure (body temperature after treatment/body temperature before treatment). Rats of two weight groups were tested. No significant correlation was found for either 130 g rats (R-Sq = 15.5%, $P = 0.132$), or 280 g rats (R-Sq = 15.9%, $P = 0.066$), when tested independently.**

Impact on physiology

As shown in Figure 6, the temperature drop after treatment was found to correlate with the dose ($P < 0.014$), although significance was found only for all rats when the data were pooled, and not for the 130 g rats or the 280 g rats alone. After open-field testing, neither body temperature, serum glucose nor serum corticosterone seemed to correlate with the dose.

Discussion

The impact of increasing dosages

Increasing gavaging volumes of $BaSO_4$ in 250 g rats showed that volumes up to 4 ml cause a spontaneous release of the test substance into the duodenum in only a very limited number of animals. Volumes of 4–8 ml represent the limit above which the pyloric sphincter opens in some of the animals. If doses are higher than 12 ml, it is unlikely that any animal will be able to keep all of the $BaSO_4$ in its stomach. Higher doses may not cause increased pain or discomfort in the stomach, because gavaged liquid flows immediately into the intestines. However, the behavioural data show that increasing doses have an increasing impact on the animals. These data are only partly supported by the physiological data, as increasing the dose resulted only in a dose-dependent decrease in immediate body temperature; after the open-field test, no correlation between body temperature and dose was found. Neither did plasma corticosterone or glucose seem to be related to the dose. Therefore, one interpretation of the data is that if the behavioural responses are caused by stress at all, this stress is probably of a short duration. If this were not so, a clear difference in stress hormone levels between gavaged and control rats should have been observed; this was not the case. The behavioural response

may result from non-stress-related conditions, such as the rat feeling heavier or less hungry after dosing. On the other hand, our observation that the surplus immediately flows into the duodenum may mean that the rats do not feel much difference in relation to stomach filling. There is no reason to believe that the rats should not all feel equally hungry, as they have approximately the same serum glucose values. Finally, exploratory behaviour is also caused by the search for water, nesting materials, conspecifics or predators and, therefore, filling of the stomach should not be able to reduce exploratory behaviour on its own. It should be noted that rearing up onto the hind legs was also reduced, although this behaviour may not be part of food searching.

Animal welfare implications

Every day, thousands of rats are dosed by gavaging in a range of studies all over the world. Many of these studies are long-term toxicological studies, in which the test compounds have little impact on the well being of the animals and the experimental procedures (ie dosing and sampling) are the main welfare factors. If these can be performed with the least possible negative welfare impact, then much will have been achieved for the welfare of these animals. It is, therefore, of the utmost importance to set standards for acceptable volumes. Such volumes should be small enough to be unlikely to cause any harm to the animal, and high enough to avoid repeated dosing of the animals. Although it is possible to repeat the dosing procedure, it would increase the stress caused by restraint during dosing, which may be more important than the stress caused by the dose volume.

Our studies have shown that doses for rats may be set higher than those given in some textbooks. In this study, we observed increasing uneasiness during gavaging in 250 g rats receiving more than 10 ml, but it is clear from the present study that this is not related to overfilling of the stomach. It might be caused by the animals suffocating from the extended duration of restraint, as some of the animals in these groups were slightly cyanotic at the end of this phase. Because we have noted, in connection with other studies, that rats being gavaged daily remain calmer during the procedure once they become accustomed to it, further studies might include 'trained' rats. Furthermore, it should be recommended not to dose rats that are too difficult to gavage. Although it is difficult to set a volume limit above which dosing is directly harmful to the animal, we observed discomfort with doses above 10 ml for a 250 g rat. This might indicate that best practice would be to keep gavaging volumes below 10 ml for a rat of around 250 g. It may be convenient to extrapolate these results into a general maximum dosage in terms of ml kg^{-1} body weight, which, calculated on the basis of 10 ml for a rat of 250 g, makes 40 ml kg^{-1}. However, it cannot automatically be assumed that a rat of 500 g would be unaffected by a dose of 20 ml. Therefore, for smaller and larger rats it might be more reasonable to recalculate this dose on the basis of metabolic weight (ie body weight$^{-0.25}$), which gives a recommended dose of 47 ml kg^{-1} for a 130 g rat (ie 6 ml), but only 29 ml kg^{-1} for a 500 g rat (ie 14 ml). Brown *et al* (2000) propose a dose limit of 20 ml kg^{-1}, and from our studies it is obvious that increasing doses above this limit has an increasing impact on the animal, although this impact is probably not very dramatic and not very long lasting. It could also be argued that doses should be kept much lower — below a dose which would not enforce opening of the pyloric sphincter in any animal. This dose in a 250 g rat would be a dose not more than 1 ml (ie 4 ml kg^{-1} body weight), as this is the only dose for which we observed no immediate gastric emptying.

Because a more viscous mixture would be less likely to leave the stomach spontaneously, the viscosity of the mixture to be dosed is also an important factor, as has been shown by others (Brown *et al* 2000). Similar considerations may apply to other physical or chemical

characteristics. This may indicate why more viscous oils can induce a corticosterone response in rats, which was not the case for the $BaSO_4$ suspension used in the present study. Brown *et al* (2000) found that some rats dosed with less viscous substances such as water or 1 per cent methylcellulose showed increased corticosterone levels, but these individuals had aspirated some of the compound into the lungs, which was not the case for the substance used here.

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