

Animal Experiments at Inveresk



**The suffering of animals in commercial contract
research and the implications for REACH**

Animal Defenders International

www.ad-international.org

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Our Background:

Animal Defenders International with offices in London, South America and the U.S., founded in 1990, represents the NAVS and LDF internationally, working on a range of animal and conservation issues including animals in entertainment, ivory, fur, animal experiments and alternatives. The National Anti-Vivisection Society (NAVS) founded in 1875, is the world's premier group on the issue – the first to alert the world to the use of animals in experiments and still in the forefront of activity on the subject today. We investigate, research, and publish scientific and technical reports on the use of animals in research, as well as educational materials. The Lord Dowding Fund for Humane Research (LDF) (founded 1974) is a department of the NAVS which provides funds for scientists conducting non-animal scientific and medical research, with an annual research spend of circa £300,000 (€435,667).

Introduction

Documents and photographs leaked to ADI provide a chilling insight into the world of contract research, where laboratories are paid to conduct animal experiments on behalf of manufacturers of products such as drugs, chemicals, household and industrial substances. All of the experiments outlined in this report took place in the Inveresk laboratories near Edinburgh.

Inveresk Research is a huge contract testing company with more than 2,500 employees working in over 20 countries. In October 2004, it merged with the even larger Charles River Laboratories based in Massachusetts, USA, with 8,000 employees worldwide.

Inveresk offers its clients dogs, monkeys, rats, mice, rabbits, pigs, guinea pigs, goats, cows, birds, and fish for experimentation and claims to be responsible for approximately 1% of all experiments taking place in the UK – over 25,000 animals every year.

The information sent to ADI includes seven reports of experiments commissioned at Inveresk which we have analysed here. Projects like this are rarely published in scientific journals, and so this report provides a unique insight into the world of commercial animal experimentation, one of the largest areas of animal use licensed by the UK government.

Our report discusses the conduct of experiments, the suffering of the animals, justification, relevance to the human situation, and the alternatives which are already available (or could be made so in the near future). We have highlighted mistakes, inadequacies of scientific protocols, and contradictions in reports of the studies. We also discuss the protection afforded to laboratory animals under the Code of Practice for the Housing and Care of Animals Used in Scientific Procedures (COP), issued by the UK government's Home Office under the authority of the Animal Scientific Procedures Act 1986.

There are serious implications for members of both the European and UK Parliaments who are now considering the EU's REACH chemical regulation proposals. It is accepted that the requirements for additional testing under REACH will result in a very significant increase in animal experimentation. This additional testing is likely to be largely taken up by contract research facilities such as Inveresk. Given the problems highlighted in this report, MPs and MEPs must seriously consider whether Europe even has the research capacity to undertake the animal testing currently demanded within REACH.

Our recommendations for a more effective and humane testing strategy under REACH have been circulated to MEPs and MPs in the report: *Keep animals out of REACH: An advanced science and technology testing strategy to replace the animal testing proposals within the REACH regulation*. This report hopes to inform parliamentarians about the real meaning of REACH – the day-to-day reality for animals.

Summary

Companies like Inveresk will undertake the animal tests detailed in the EU's REACH chemicals regulations. Inveresk is already offering to: *"Help chemical industry clients to comply with mandatory and voluntary chemical control schemes including European requirements for new and existing chemicals, the new European REACH system..."*

Before ministers and MEPs commit to millions of animal experiments for REACH, they need to question the laboratories, the science, and the nature of animal tests. The Inveresk reports show:

- Extreme animal suffering and side effects in procedures
- Miscalculations in dosing resulting in severe suffering, death and

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premature termination of studies.

- Researchers running out of the test substance, half way through a study.
- In one experiment dogs came from two different suppliers, some from the USA.
- A test substance passing its expiry date before the end of the experiment, whilst dosing of the animals continued.
- At least three cases of animal tests being conducted when human studies were already underway. And animal results ignored – human studies continued after adverse animal results.
- In two experiments, test substances were accidentally pumped into dogs' lungs instead of their stomachs.
- An inability to differentiate the effects of the test substance from those caused by the dosing method or lab husbandry:
In two tests on the same drug, salivation was attributed to the drug on one study and to the procedure in the other.
- Dogs given the wrong dosage of test substance.



The implications for REACH

Under the REACH proposals, around 30,000 chemicals produced in high volumes will need to undergo risk assessments and testing for their effect on humans and the environment. This will mean potentially millions of animal tests. Although the species specified in the REACH testing protocols are principally rabbits, rodents and fish the prospect of laboratories such as Inveresk undertaking such a huge testing programme is cause for concern, especially in light of the evidence contained here.

It is our experience that the larger and more expensive the laboratory species, the greater the care taken. Therefore if dogs and primates can be allowed to suffer in badly managed and poorly considered tests as described here, there is likely to be even less care with rodents. This is exemplified by the horrific experiment described later, where rats choked to death on paint.

As mentioned earlier, this report highlights where animal tests have been repeated; where mistakes are made and animals given incorrect doses of experimental substances and where animal tests using the same species and the same product cannot produce results that provide sensible data for human use.

If the contract research companies across Europe are to significantly increase the amount of research they are undertaking in order to produce data for REACH, we believe there will be serious implications for both animal welfare and the data being generated.

There has been much concern during discussions about REACH over potential duplication of animal testing. This has been addressed in the proposal itself but enforcement remains a concern. In this report, we show how the UK Home Office was apparently unable to even delay (let alone prevent) a dog test, when a similar test for the same drug was already underway. The dosing levels for the second experiment were meant to be informed by the first experiment – but it was started a month before the first study was concluded. Furthermore, like other tests in this report, the animal tests were conducted after trials on human patients had begun.

In our report *Keep animals out of REACH: An advanced science and technology testing strategy to replace the animal testing proposals within the REACH regulation*, we provide details of advanced techniques which could replace animal tests. The widespread adoption of such techniques would prevent animal suffering and would generate reliable and consistent data across Europe. Such techniques could be utilised by the contract testing bodies that are likely to be called upon to meet the demands of REACH.

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Project 571898: dogs force-fed experimental psoriasis drug

Twice a day for a year, a tube was pushed down the throats of 48 beagles and VX-497 (a potential psoriasis drug) or a control substance was pumped into their stomachs. The drug was being tested for US company Vertex Pharmaceuticals between 1998 and 1999.

Force feeding animals via a tube into the stomach is known as 'gavage dosing'. Such techniques are usually distressing to conscious animals² as is the restraint required to perform the procedure⁷. Inveresk's experimental protocol (the document outlining the experimental procedure) classifies the probable severity limit of this procedure as 'moderate'.

The dogs were divided into 6 groups; 4 with equal numbers of males and females, and 2 single-sex groups. Four groups were dosed with varying concentrations of VX-497. The top dose groups were the single sex groups; the males were given the highest concentration. Two of the mixed-sex groups were controls.

Since the dosing procedure itself is highly distressing, there seems no justification for as many as 16 animals being subjected to it simply to act as controls. Indeed, Inveresk's protocol for chronic toxicity studies in dogs specifies that one control group is used. Furthermore, a recent paper on design of preliminary pharmaceutical toxicity studies has questioned the need for control groups at all³.

The dose levels for this test were based on a preliminary Maximum Tolerated Dose (MTD) study, a 28 day toxicity study and a 13 week with 4 week recovery toxicity study "*in the same species*" (Inveresk Project Numbers 566956, 567106 and 568141 respectively).

Following dosing, the dogs were observed at intervals each day for reaction or signs of ill health. Body weight, food consumption, heart activity, blood samples, urine samples and faecal samples were analysed, as well as eye examinations. At the end of the test the animals were killed.

Five of the dogs in the top dose group "*were given dosing holidays during the study due to low body weight and poor food consumption*" and the dose for all the animals in the group had to be reduced.

Experimental blunders cost the lives of two dogs before the end of the test. Following dosing, one dog cried out and salivated profusely, with red material foaming from the mouth, a low heart rate, laboured breathing and flickering eyes. Another became distressed and salivated profusely immediately after being dosed. "*It was suspected that both animals had been lung dosed*". Both dogs were killed. Post mortem findings confirmed that the product had been pumped into their lungs instead of the stomach.

There was a high incidence of vomiting and regurgitation, salivation, and of loose/liquid faeces in top dose groups. But these signs were also seen in the lower dose groups and even the controls.

As the experiment progressed, the dogs suffered: green/yellow/red/dark purple/orange faeces; diarrhoea; loss of appetite; vomiting; regurgitated food; subdued behaviour; scabbing; abrasions; scratches; discharge from eyes; lymph node swelling; hair loss and discolouration; swollen joints; bloody/clear/brown vulval discharge; enlarged vulvae; salivation; thinness; bleeding gums; puncture wounds; lameness; sores; warts; skin reddening and weight loss.

According to the protocol only mild or sub-clinical effects are anticipated in studies of longer than 6 months duration (such as this one). During this study severe clinical signs were seen throughout the dosing period (total of 52 weeks). Post-mortem findings included: Heart lesions; pneumonia; ovarian cysts; haemorrhaging in the stomach and intestines; damage to the pituitary gland; congestion in the rectum and bladder.

Over half of the animals had injuries from fighting with records of bite/fight wounds for 26 of the 48 dogs, and two others suffered abrasions or scratches.

Critical Review

The 'no effect' level established by this study was considered to be 4mg/kg. This is "*apart from the mild clinical signs of occasional vomiting and salivation*".

However, many apparently serious side effects, including vomiting, salivation, loose/liquid or red faeces, enlarged vulvae, and bloody discharge from the vulva were seen after dosing at the 4mg/kg level. It is stated in the report that "*this study is part of animal toxicity experiments designed to provide information which will allow the material to be used safely.*" We would question the ability to extrapolate any of this dog data to people with any confidence. It appears that certain symptoms were ignored

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when establishing the “no effect” level – but rather than this being a concern, the Inveresk staff appear to consider it to be an inherent imprecision in the test.

The procedure itself had an impact on the animals. This is demonstrated by the fact that the control animals suffered vomiting, loose/liquid faeces and salivation. This may have been due to feeding the dogs just 30 minutes before oral gavage dosing.

Even between individual animals there was a large degree of variation in the effect of the test substance on weight loss and food consumption.

Gender and individual differences between the dogs in terms of the systemic exposure of VX-497 are highlighted in the report. Systemic exposure refers to the exposure of different internal body systems (e.g. reproductive, digestive etc.) to a drug¹. There was a tendency for the females to be more affected by the drug than the males; however some individual animals were more affected than others. The potential for problems of species differences with this drug are apparent¹.

Any adverse findings which could not be attributed to treatment were said to be representative of the background abnormalities seen in beagles at Inveresk, indicating the possibility that dogs at Inveresk are not in good general health.

Yet another, unanticipated variable was added when five of the top dose group animals had to be given “dosing holidays” at several different points during the study due to low body weight and poor food consumption. A decrease in food consumption in all top dose group animals required that the top dose levels were reduced 44 weeks into the experiment (although adverse effects were apparent soon after the start of the test). Considering that the dosages were based on three previous experiments in the same species, if it was not possible to extrapolate the data between experiments on the same species, one can have little confidence that the data from this experiment is of any value in people.

To add just a little more confusion to this experiment, the expiry date on the test material was a month prior to the end of dosing, which means that the final month of dosing was with an expired product.

A study to determine the MTD of VX-497 in rats (Inveresk Project 492654) was underway during the last two months of the dog study. 75 pregnant female rats were oral gavage dosed with VX-497 for approximately 5 weeks.

Adverse clinical signs included: Brown/yellow staining of head and body; bald areas/hair loss; salivation; crackling/wheezing respiration; red discharge from area around genitals; hind feet swollen and unusable; rolling gait; unkempt and greasy coat; subdued behaviour; red staining around eye; mouth ulceration; firm and irregular abdomen; weight loss; high stepping gait; hunched posture; decreased faecal output; limping. Post-mortem findings of the baby rats included: Small and opaque eyes (if present at all); shortened tail; distended bladder; brain tissue reduced in size and abnormal in shape (if present at all); flesh protrudence from both sides of mouth; shortened lower jaw; hole in abdomen; protruding tongue; missing bones.

Why an experiment likely to cause such suffering was authorised by the Home Office for a start date just weeks before the dog study was due to conclude, is a matter of grave concern.

The experiment on the 48 beagle dogs ran from November 1998 to December 1999, whilst the drug was already being taken by human volunteers in the UK.

In January 1998 it was announced that the Vertex Pharmaceuticals new drug VX-497 – inosine monophosphate dehydrogenase (IMPDH), would commence Phase I Clinical trials in the UK⁴. At this time it was being tested for the treatment of autoimmune diseases. In September 1998⁵ (still prior to the start of the dog study), Vertex announced that IMPDH was in Phase II Clinical studies in patients with Hepatitis C infection. In addition to treatment for autoimmune diseases such as psoriasis, IMPDH appears to inhibit the growth of RNA and DNA viruses, including viruses closely related to hepatitis C.

In January 1999 (with the dog study approximately a sixth of the way through, and the animals showing some serious side effects), IMPDH Phase II Clinical studies began in patients for the treatment of psoriasis⁶. This was months before the Inveresk rat experiment had even started.

This directly contradicts statements over the years by the government and the vivisection industry that products must be tested on animals *before* they can be given to people.

It also contradicts Inveresk’s own publications which state: “Almost all animal tests carried out at Inveresk are in the field of



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Regulatory Toxicology. This is work required by UK and other governments to demonstrate the safety of new materials before they will be given a licence to be sold to the public or tested in human volunteers." [our emphasis].

1 U.S. Food and Drug Administration Guideline for Industry (1995) Toxicokinetics: The Assessment of Systemic Exposure in Toxicity Studies (<http://www.fda.gov/cder/guidance/ichs3a.pdf>)

2 Wallace J et al. (1990) *Laboratory Animals* 24: 97-130

3 Smith D et al. (2005) Optimising the design of preliminary toxicity studies for pharmaceutical safety testing in the dog. *Regulatory Toxicology and Pharmacology* 41: 95-101

4 Vertex Pharmaceuticals Press Release: 13th January 1998

5 Vertex Pharmaceuticals Press Release: 21st September 1998

6 Vertex Pharmaceuticals Press Release: 21st January 1999

7 Beerda, B. et al (1999) *Physiology & Behaviour* 66(2):633-642

Project 573146: urinary incontinence drug force-fed to dogs

Between December 1999 and January 2000, 24 beagle dogs were dosed daily for 28 days via oral gavage direct into the animals' stomachs to test an incontinence drug for Kyowa Hakko Kogyo Co. Ltd., of Japan.

The dogs were divided into 4 groups of 6 animals (3 males, 3 females). The animals in the first group were controls. The other groups were each given a different concentration of KW-7158. "Doses were administered using a rubber gavage [into the stomach] attached to a plastic graduated syringe."

"All the animals were examined for reaction to treatment at frequent intervals during the normal working day." Body weight, food consumption, heart activity, blood pressure, blood samples from the jugular vein, urine samples and faecal samples were all analysed at intervals throughout the study. The eyes were examined via ophthalmoscopy. The dogs were deprived of food and water overnight to facilitate collection of urine and faecal samples. The 'metabolism' cages used for this generally have a mesh floor (to enable excrement to fall through for collection), and are bare and uncomfortable. According to the protocol for this experiment, animals can be held in metabolism cages for up to 24 hours. All animals were killed after 28 days.

Some of the dogs showed an increased heart rate, particularly those at the highest dose level. However, increased heart rate has been linked to the stress response in dogs¹ and stressors such as noise, transport and restricted housing conditions have been reported to affect heart rate². Again the use of live animals and employing procedures such as gavage dosing and equipment such as metabolism cages all introduce variables which affect subsequent results.

As in the experiment discussed previously, the control group also showed adverse effects including loss of weight and salivation. Once again at Inveresk there were injuries from fighting in all the groups of dogs.

In the low dose group there was weight loss, and in the males, a bite wound, bruising and swelling around the penis, white frothy vomit. The females suffered skin reddening, white frothy vomit, salivation, puncture wound to top left forelimb due to fighting, wounds to tail, right shoulder scratched and bruised, loose faeces.

In the inter dose group, animals suffered a reduction in body weight; increased heart force; skin reddening. Males had bite wounds (including bite wounds to inside of mouth), they were thin and subdued, and salivated. The females suffered enlarged mammary glands and large amount of liquid faeces.

The dogs in the high dose group suffered the largest reduction in body weight paralleled by a marked decrease in food consumption; increased heart rate; increased heart force; decreased blood pressure; reddening of skin; large amount of food regurgitation; large amount of brown vomit; salivation. The males had scratches on muzzle; bite wounds to ear; loose faeces; the females were thin, and salivating. Post mortem findings were that all of the animals suffered increased adrenal gland, liver and heart weight.

The dose levels chosen for this experiment were based on "evaluation of the results obtained from MTD studies performed at Inveresk (Inveresk Study No. 573130) and at the Sponsor's laboratories in the same species". The purpose of using such studies to set dose levels is "in order to limit the effects of treatment...to typically mild, or occasionally moderate, severity." Yet the symptoms endured by these animals cannot be described in this way. This means that the experimenters could not set accurate dose levels based on their own experiments in the same species – there can be little confidence that such results can be extrapolated to humans.

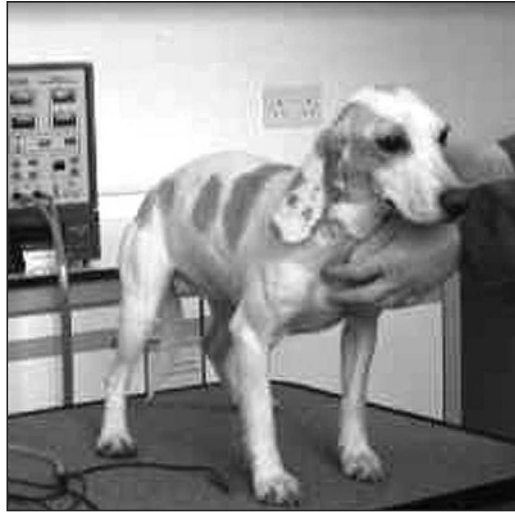
Critical Review

The animals in this study suffered more than necessary because the the incremental increase in dose levels was very high – a factor of 10 each time. Not surprisingly, this resulted in increasingly severe adverse reactions each time the dosage increased. Had the incremental rises in dose levels been smaller, or had the dose levels been titrated, then it is likely that the suffering would have been greatly reduced.

Dogs were offered food immediately after dosing. This means that the cause of the loss of appetite and commensurate weight loss cannot be clearly attributed to the dosing method or a reaction to the drug, since the dogs had a stomach full of test substance when offered food.

Both females and males in the group with the highest concentration of the drug lost their appetite. For the females, the food was wetted and reoffered, but this was not done for the males. This highlights yet another inconsistency in the treatment of the animals and another variable which could affect the data.

Vomiting noted throughout the study *“could not be unequivocally related to treatment with KW-7158.”* Indeed there are a range of factors that may have made the dogs vomit. Stresses caused by environmental conditions have been shown to produce digestive disturbances in dogs, including vomiting³. The behavioural warning signs of stress in dogs include intermittent vomiting with no discernable medical cause, commonly attributed to being kept in an unfamiliar environment and exposure to unfamiliar sights and sounds⁴. It has been said that dogs exposed to conditions which induce social and psychological stress and cause vomiting, should be removed from such conditions⁵. However, despite repeated fighting and unexplained vomiting in the Inveresk dogs, no remedial steps were taken nor were there recommendations to do so.



Despite the considerable suffering of these dogs and the adverse reactions they endured, the drug progressed to human clinical trials in both the USA and Europe⁶.

1 Boxall J et al. (2004) Modern concepts of socialisation for dogs: Implications for their behaviour, welfare and use in scientific procedures. *ATLA* 32: 81-93
2 Bearda B et al. (1997) Manifestations of chronic and acute stress in dogs. *Applied Animal Behaviour Science* 52 (3-4): 307-319
3 Brugere H (1991) Physiopathology of stress-induced conditions in sporting dogs. *Recueil de Médecine Vétérinaire* 167 (7-8): 635-645
4 Pet Health Council: Stressed Pets Leaflet (http://www.pethealthcouncil.co.uk/leaflets/Stressed%20Pets_leaflet.doc)
5 Nutrition and Stress in Dogs (http://www.animalhealthcare.ca/contents/content_careguides2.asp?id=79&keywords=nutrition+and+stress)
6 Kyowa Overseas Research and Development Activities (http://www.kyowa.co.jp/eng/company/iyaku/overseas_r_d.htm)

Projects 573869 & 574841: Two dog tests, one anti-depressant

Twelve beagles were force fed a potential anti-depressant drug for Neurosearch A/S of Denmark (September 1999 – April 2000). However before this experiment was even completed, the UK's Glaxo (which held a licence from Neurosearch for the drug) began another experiment using forty dogs (March 2000 – June 2000). The first experiment was meant to determine the doses for the second, but it had not been completed when the second experiment commenced.

The drug caused the dogs to suffer reddened ears and gums; loss of weight; dull coat; stereotypic behaviour; agitation; reddened eyes with watery discharge; dilated pupils; irregular heartbeat; excess saliva and an intolerance to light – but this was a moot point by then – the drug was already being taken by people. It had progressed to clinical trials before the dog tests had begun.

In the Neurosearch experiment, 12 beagle dogs (bizarrely obtained from two different dealers thousands of miles apart in the UK and USA) were force fed the anti-depressant or control substance daily by oral gavage. The study consisted of two parts: Part A (81 days) in which 4 dogs were used and Part B (102 days in total) in which 8 dogs were used.

Part A of the study involved daily dosing with an increase in the concentration of the drug (code NS 2389) every three days. At the lowest dose, no abnormal clinical signs were noted, but as the dosing concentration increased, the dogs were seen to have reddened ears and gums. By the maximum dose, some animals appeared thin with dull coats, and all of the male dogs displayed stereotypical behaviours.

Part B of the study consisted of 5 phases. The organisation of this part of the study was not in accordance with an experimental protocol but rather followed this sequence because preceding phases failed. Subsequent phases were conducted because they were seen as necessary in order to obtain meaningful results; no useful information could be gleaned from previous phases. It would seem the protocol here was make-it-up-as-we-go-along.

Phase 1: Two dogs were given the maximum tolerated dose (MTD) determined by Part A. The dogs became agitated, had reddened eyes with a watery or mucoid discharge and made repetitive movements. They also suffered dilated pupils, reddened mouth and gums, excess saliva and an intolerance to light. These severe reactions were thought to be the result of sudden exposure to such a high concentration of the drug. The dogs in Part A were thought to have built up tolerance to the drug; hence the effects seen in the Part A animals were less severe.

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Phase 2: Because of the severity of reactions in Phase 1, two more dogs were dosed with escalating concentrations of the drug, commencing at lower levels. However even this caused the animals to suffer reddened ears and gums, subdued behaviour, and one dog vomited.

Phase 3: The dogs were left undosed for ten days and then dose escalation began again. This time, the animals were given a lower concentration than the starting dose level of phase 2. As dose concentrations increased to just below the MTD determined by Part A, vomiting (which had already been seen at the outset), subdued behaviour, dilated and unreactive pupils, repetitive movements, agitation and an irregular heartbeat were seen in the dogs. The severity of reactions increased with dosage.

Phase 4: Four more dogs (from Marshall Farms, U.S.A) were given escalating titrating doses over 30 days. This was in an attempt to avoid the effects of NS 2389 and glean some meaningful results. During this period however, the dogs experienced dilated and unreactive pupils, reddened ears, mouths and gums, repetitive movements, agitation, loose/liquid faeces, salivation and watery discharges from the nose and mouth.

Due to the severity of some of the reactions seen in the dogs, it was deemed to be necessary to titrate the doses of NS 2389 to avoid such acute effects. However, this conclusion was only reached after 8 dogs had been subjected to escalating doses that were not titrated, and suffered as a result. There were numerous earlier opportunities to acknowledge that titrating doses would be necessary, especially since there were 3 separate experimental phases involving small groups of animals. After each of these, it was acknowledged that effects were “too severe” or doses were “too high,” and that negative findings were “considered to be associated with dosing of NS 2389...without titration.” Titrating doses of substances ensures that the optimum dose is determined between the amount that causes the desired response and that which results in negative effects. Titration methods are regularly used to administer drugs to humans, such as the use of opioids to provide pain relief⁸, where titrating doses provides the balance between respiratory depression and effective pain relief⁹.

Phase 5: The same four dogs were dosed with the MTD (determined in Part A) for a fixed dosing period of 14 days. During this period, the dogs experienced reddened ears, mouths and gums, weight loss, dry flaky skin, dull dry coats, mucoid discharge from the eyes, increased heart rate, agitation, salivation, matted hair around the neck and tremor upon handling.

The animals were killed and post-mortem examination revealed abnormal dark fluids in the small intestine, inflammation of the intestine, congestion in the liver, and encrustation and inflammation of the eyelids. “Other findings noted were considered to be typical of expected spontaneous findings in young beagles at Inveresk.”

A month before the test for Neurosearch had completed, Inveresk started a six week toxicity study of the same drug (NS 2389) for Glaxo Wellcome (now Glaxo SmithKline) using 40 more dogs. The aim was to assess the toxicity associated with repeated oral gavage dosage of NS 2389 to beagles for approximately 6 weeks, and to assess the reversibility of any effects following a 2-week recovery period.

The report notes: “The oral gavage route is one of the proposed routes of administration to man.” We presume they mean that NS 2389 drug will end up in patient’s stomachs but not get there by being pumped down a rubber tube forced down their throat.

Three groups of 4 male and 4 female dogs received different concentrations of NS 2389 for 6 consecutive weeks (fixed dosing phase). Eight more dogs were controls. The control and top dose groups included two extra males and females which were kept for a 2-week post-dosing recovery period. These animals received increasing doses of the drug, with the dose rising every four days for four weeks in order to build up tolerance. All of the animals were then killed.

The dogs suffered various symptoms including: Dilated and unreactive pupils; reddening of the ears, including controls; mucoid or watery discharge from the eyes observed in a number of animals; decreased body weight and food consumption during the escalating dosing phase, and increased heart rates. A number of opacities were observed in the cornea and lens of animals across all dose groups. Post-mortem findings included: Increased adrenal gland weight; decreased heart and spleen weights; Increased liver weight. In recovery animals, spleen and adrenal weights increased compared with controls. Nine animals across all treated groups had reddened or dark red/mottled lung lobes related to fluid accumulation and congestion and a variety of inflammatory lesions in the lungs.

There were two significant errors in the study for Glaxo Wellcome. Two dogs were mis-dosed and received approximately 30% less than the intended dose volume, and repeat blood samples had to be obtained from five dogs because of “accidental spillage” on different occasions.

It is claimed that because the animals which were mis-dosed were in the control and low dose groups, and mis-dosing occurred for 4 days only, “this deviation is not considered to have compromised the study.” This again highlights the lack of precision in such animal tests.

Critical Review

The Glaxo Wellcome study was claimed to have been “based on the findings of an earlier study (Inveresk Project No. 573869).” But project 573869 was the study for Neurosearch and we know that this still had a month to run when the Glaxo Wellcome study started – they didn’t wait to finish the first dog test.

The authors of the second study noted that the 20mg/kg dose level administered to the dogs in the Neurosearch study had been too high, and so they decided that 18mg/kg would be a suitable concentration to escalate to. There appears no clear rationale for this. Indeed, dogs in the Neurosearch study on much lower doses than this endured dilated pupils, subdued behaviour, vomiting, reddened ears, mouths and gums, and repetitive movements. The authors of the Neurosearch study stated that only “the clinical signs of repeated movement, dilated pupils and agitation are considered to have been a pharmacological effect of NS 2389.” The other effects observed are therefore regarded as adverse and avoidable.



Comments in the report for the Glaxo study contradict those in the Neurosearch report. In the Neurosearch study, it is stated that the recorded increase in the heart rate of the 4 dogs used in the final phase of the study is “not considered to have been due to treatment” and that any variance in heart rate “could be due to increased physical activity immediately prior to measurement.”

But in the Glaxo study where “increased heart rates were observed in top dose animals following dosing”, the increased heart rates “may be related to stress associated with administration of NS 2389”. Certainly dogs that are restrained and stressed have been shown to have pronounced increases in heart rate¹. It is unlikely that increased heart rate could be attributed to a recent peak in physical exercise, since the animals were housed in cages and were only exercised for 30 minutes once a week, and furthermore, heart rate recordings were taken frequently during the study.

This highlights the difficulties of attempting to make measurements of any precision based on frightened animals. Behavioural parameters of stress in dogs can include body shaking and trembling^{1,2}. High levels of body shaking have been observed in dogs by simply placing them in the presence of an unfamiliar researcher, without any subsequent treatment³. Such high levels of body shaking have been associated with acute stress³.

So although the authors report that “the clinical signs of repeated movement, dilated pupils and agitation are considered to have been a pharmacological effect of NS 2389,” these could also be indicative of stress and poor welfare². Behavioural changes associated with chronic stress (such as incidents of repetitive behaviour) may be exacerbated by individual rather than group housing². During this study, the dogs were housed in individual pens.

During the final phase of the Neurosearch study, salivation was seen, but was regarded as an isolated incident and “not considered to have been due to treatment.” Yet excess salivation had already been seen in an animal which had received the highest dose of the NS 2389 and salivation had been listed in the pharmacological signs which were considered “too severe” when dosing naïve animals.

Having already established that tolerance to the drug affects results, two of the dogs were still re-used. Having been given with a higher dose, they were then given a lower dose. Although there was a 10-day period in which they remained undosed, tolerance to the higher dose levels would already have been established. Therefore, it is likely that there would not have been a significant reaction to the drug until higher levels were administered. This has implications for the Maximum Tolerated Dose which this study is trying to determine and therefore the longer term toxicity studies which the MTD is used to set appropriate dose levels for. All of the results are incorrect; no abnormal signs were seen in these re-used dogs until 12mg/kg were administered but when the 4 naïve dogs from Marshall Farm were administered with titrating dose levels of 8-10mg/kg, they exhibited a markedly more severe response. Therefore, the MTD determined is misleading. Once again, the experimental ineptitude of the scientists has caused more dogs to suffer than was necessary to achieve results.

A study was conducted in rats to assess the toxicity and effects on male fertility of NS 2389 (Inveresk Project Number: 492607). This study was completed, and results obtained, before Project Number 573869 began. 210 rats were dosed with NS 2389 via oral gavage and the study lasted for approximately 12 weeks. Two animals died prematurely.

The rats suffered abnormal colouring (red/purple) of ears and tail, agitation, brown/yellow staining on head, neck, nose, muzzle and tail, black staining around eyes, broken teeth, hair loss, abnormal vocalisation, swollen muzzle, irregular and slow respiration, swollen eyelids, black swelling and scabbing on head, soft faeces, subdued behaviour, salivation, wheezing,

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hunched posture, unkempt coat, hair erect, weight loss and decreased food consumption. The post mortem findings discovered that the animals had lesions; staining of skin; hair loss; reddened lymph nodes; scabs; reddened ears and stomach; speckled and spongy lungs; small and flaccid testes; enlarged thymus; froth-filled trachea; abnormal masses in thoracic cavity. It would appear that regardless of adverse effects in one species, testing simply moves on to the next.

The pharmacological effects of the NS 2389 as determined by the studies included *“repeated movement, dilated pupils and agitation”*. Since these appear to be the last things someone taking an anti-depressant would want to experience, one might assume that would be the end of NS2389.

In fact seven months before their dog experiment, in February 1999, Neurosearch received approval to begin Phase II clinical studies of NS 2389 on human volunteers and two of these studies were completed in that year. Phase II involves healthy human volunteers taking part to evaluate the effectiveness of a drug and potential side effects. This typically follows Phase I clinical studies in which human volunteers are tested to determine the safety and correct dosage of the test product in humans.

In January 2000, Neurosearch concluded a licence agreement with GlaxoSmithKline to develop and market the antidepressant compound NS 2389. So they would not have been excluded from data from the human trials, yet still instigated yet another dog test. Neurosearch and GlaxoSmithKline terminated the development of NS2389 in 2001 as their additional safety studies *“did not produce sufficient documentation for continuing the development”*.

Modern solutions

There are fundamental structural and physiological differences between dogs and humans which make dogs an unreliable model for prediction of likely effects in humans. Furthermore, in the laboratory situation this problem is exacerbated by the biochemical changes that occur in animals in the laboratory environment. Stressors include housing and environmental conditions, withdrawal of food and water, handling, blood sampling, and the experiments themselves – whether surgical intervention, preparation or the effects of experimental products. All of these factors can affect results.

The UK's Laboratory Animal Science Association has admitted that: *“Manipulations or the application of substances to the eyes, feet, nasal passages, respiratory system or by oral gavage, are usually distressing to conscious animals even when pain is not involved”*⁴.

The difficulties of extrapolating the results from animal tests to humans are well known:

- *“The dog is a poor model for man, and, while the pig has some advantages, the best model for the early evaluation of drug absorption in man is man”*⁵.
- *“To date, guidelines for neurotoxicity testing involve animal-based models only and are therefore expensive and time consuming. Besides, some chemicals (e.g. antibiotics and peptides) may be human specific (not detectable by animal models)”*⁶.
- *“Selection of an animal species for an experiment is dependent on the type of study, but the rat, the mouse and the dog are the species most frequently used in toxicity testing. Since variation between animal species and man with respect to kinetics and metabolism of xenobiotics is largely unknown, this choice is not based on scientific rationale”*⁷.

There are a number of readily available alternatives to the use of animals in drug development and toxicity testing. Advanced techniques which do not rely upon animals, and concentrate on methods more relevant to humans are the way forward.

Some of the examples listed below are still awaiting validation for use in Europe, although some scientists have argued that the process of validation is too drawn out (especially as compared to animal tests which have never been similarly validated):-

Toxicity Testing:

DEREK (Deductive Estimation of Risk from Existing Knowledge: a computer-based system whereby toxicity predictions are made on the basis of a series of 'rules' relating chemical structure to possible toxicity. The user 'draws' a two-dimensional diagram of a given chemical structure on the screen (for evaluation of large numbers of chemical structures automatic processing can be carried out), which initiates an automatic check against a knowledge database containing information on the toxicity of chemicals in biological systems, taken from the published literature. Any 'structural alerts' (i.e. possible toxicological hazards) for a given chemical structure are then displayed on screen, along with the toxophores (i.e. the part of the molecule carrying the poisonous principle) and their toxic effects (e.g. skin sensitisation, mutagenicity, carcinogenicity etc). Also displayed are the relevant references from the biomedical literature.

MTT *in vitro* Assay: The purpose of this test is to assess the neurotoxicity potential of substances. Cultured differentiated cells are exposed to chemicals of different concentrations for varied periods (1h-24h), after which the tetrazolium salt, 3-(4, 5-dimethyl-thiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) is added to the cells. Mitochondrial activity is then measured by the ability of the cells to reduce MTT to an insoluble formazan product. The MTT assay can be used to measure the metabolic activity of cells – indicating any loss of ability to reduce MTT after exposure to test chemicals. Such loss is important, as the ability of cells to reduce MTT provides an indication of mitochondrial integrity and activity, which is interpreted as a measure of cell viability.

Neutral Red Release Assay (NRR): This is based on the principle that if cells in culture are preloaded with vital dye (neutral red [NR]) and then exposed to a toxic substance, the amount of dye released from the cells will reveal the degree of membrane damage. The result of the test is expressed as the concentration of the test substance that gives rise to the release of 50% of the NR from preloaded cells (the NRR50).

Neutral Red Uptake Assay (NRU): This technique measures the effect of a test chemical on cell growth and viability during a 48-hour exposure period. Cultured cells are exposed, first to a test chemical, and then to neutral red dye. Retention of red dye indicates cell viability – damaged cells retain less dye than unaffected ones. The amount of dye retained is measured spectrophotometrically.

Silicon Microphysiometer Test: Uses a sensor that measures the effects of test substances on the metabolic rate of cultured cells. The light-addressable potentiometric sensor allows indirect measurement of real-time changes in cellular metabolism, providing monitoring of the rate at which living cells inside a flow-through chamber excrete acidic metabolites as a normal part of energy metabolism. Irreversible reductions in cell metabolism reflect cytotoxicity and cell death. The technique can be used in the assessment of acute toxicity, and has been shown (using human cell lines) to produce IC50 values (i.e. the concentration of a chemical or compound that causes a 50% inhibition of some specific cellular function or response) that, for 50 chemicals, were more accurate predictors of human toxicity than equivalent toxic blood concentrations derived from rodent LD50 values.

Drug Development:

Cultured Human Hepatocytes using a Bioreactor: This procedure can be used for both toxicity testing as well as to contribute toward the development of a new drug. Primary cultures of human hepatocytes are treated with a substance. The bioreactor is constructed as an interwoven 3-dimensional network using three separate capillary systems. Primary liver cells from human volunteers are inoculated into bioreactors and cultured in continually changing medium for up to 35 days. Biochemical parameters are assessed (enzymes, hormones, albumin, urea, gases, glucose etc.).

Microdialysis: *In vivo* microdialysis measures the chemical composition of the interstitial tissue fluid, i.e. fluid to which cells and other target structures are directly exposed. Microdialysis is a sampling tool and needs to be linked to an appropriate analytical instrument. However, with suitable analysis, virtually every soluble molecule in the interstitial space fluid can be measured by microdialysis. Samples are obtained by means of a semi-permeable membrane at the tip of a probe. No special skills are required to insert a microdialysis probe. In soft tissues and the skin, the procedure can be managed by any health professional and is no more painful than the placement of an intravenous catheter. After about 10 minutes, most subjects do not report any pain at all. The probes are usually left *in situ* for several hours, but they can be left in place for up to several weeks. Studies of people during exercise are possible with flexible probes. Reports detail microdialysis in human tissue such as brain, heart, lung and solid tumours.

Microdosing: Tiny amounts of new drugs are safely given to human volunteers.

QSAR – Quantitative Structure Activity Relationship: These programmes relate statistically the biological activity of a series of compounds to their physicochemical and/or structural properties. As such they provide predictive models to estimate the biological activity in question. The majority of work, stretching back over the century, in this field has focused predominantly on the optimisation of drug activity and the prediction of toxicity. These programmes are already being used by some regulatory authorities around the world.

1 Beerdia B et al. (1998) Behavioural, saliva cortisol and heart rate responses to different types of stimuli in dogs. *Applied Animal Behaviour Science* 58: 365-381

2 Beerdia B et al. (1999) Chronic stress in dogs subjected to social and spatial restriction: behavioural responses. *Physiology and Behaviour* 66 (2): 233-242

3 Beerdia B et al. (2000) Behavioural and hormonal indicators of enduring environmental stress in dogs. *Animal Welfare* 9 (1): 49-62

4 Wallace J et al. (1990). Laboratory Animal Science Association Working Party. *Laboratory Animals* 24: 97-130

5 Wilding I. *Scrip* (2001).

6 Hernandez M., Kisaallita WS (1996). University of Georgia, Athens. *Toxicology in Vitro* 10: 447-453

7 Van der Sant JJM and Feron VJ (1996). TNO Nutrition and Food Research Institute, Zeist, Netherlands. *Alternatives to Laboratory Animals* 24: 173-178

8 McQuay, H. (1999) *The Lancet* 353:2229-2232

Gould, TH et al (1992) *British Medical Journal* 305:1188-1193

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Project 574574: asthma drug tested on monkeys

In a test categorised with a 'moderate' severity limit, a cannula (small tube) was inserted into the leg vein of 16 cynomolgus macaque monkeys where it remained so that they could be dosed with an experimental asthma drug.

Each day for for 14 days, the animals received an intravenous injection (despite that it was intended that the drug should be inhaled by humans). The animals were then killed. The customer was Astra Zeneca and the animals were supplied by Shamrock Farms (now closed).

The dose of the drug was based on previous tests in the same species. The MTD and other factors such as the anticipated therapeutic dose, were known. Following injection, the monkeys were observed at regular intervals throughout each day for any signs of ill health or reaction to treatment. The animals' eyes were examined and body weight, food consumption, heart activity, blood pressure, blood samples and urine samples were all analysed at intervals throughout.

In order to obtain samples for analysis, the monkeys were deprived of food and water, and will have suffered through human handling, and discomfort due to the procedures themselves. For urine collection the monkeys were deprived of food and water overnight, and deprived of food overnight prior to blood sampling.

The insertion of the cannula required for the intravenous infusion was conducted under general anaesthesia, however the animals would have suffered some discomfort afterwards, and throughout the 14 days.

From approximately 5 minutes after dosing, all of the animals suffered reddening of the face and lips; this increased with dose levels, in both incidence and length of time. In addition, they experienced a range of symptoms including diarrhoea, swelling in the stomach, redness of feet and hands, white pigmentation on the feet; the males' testes increased in weight and they suffered red and swollen penises and scrota. The monkeys were reported to be subdued and hunched on their perches; body tremors were seen in one; another did not use its right leg throughout the test, apparently due to "*an injury*"; females suffered abdominal and umbilical hernias. All of the monkeys lost weight; both food consumption and heart rate fell.

Control animals (which were not given the experimental drug) were noted to have suffered from diarrhoea, indicating that the animals were not in good health, or were suffering from poor husbandry.

It is not surprising that the monkeys suffered a loss of appetite and reduction in bodyweight, since food was offered immediately after a drug had been pumped into their bloodstream.

Critical Review

The report of this study admits that the client, AstraZeneca, was in possession of information from previous experiments on cynomolgus monkeys, stating that: "... has indicated that the test compound may affect the cardiac function and produce pericardial effusion in cynomolgus monkeys when given intravenously or via inhalation". It is not stated whether this information was from previous experiments, or taken from the scientific literature at the time. However, the use of cynomolgus monkeys has been criticised as being the most misleading laboratory animal model for the study of human cardiotoxicity¹ (i.e. toxic effects on the heart), yet this is one of the key questions that the test was to address.

Furthermore, the report states that the experimental drug "...is intended for inhalation administration for the treatment of asthma". However, the drug was given to the monkeys directly into the bloodstream, through the catheter into the leg vein. Yet it was already known that a similar asthma drug developed by AstraZeneca gave different results after administration by intravenous infusion compared with administration by inhalation³.

Inhalation and intravenous routes of drug administration have been found to differ in terms of the side effects they cause. A high incidence of side effects was observed with the intravenous administration of salbutamol (another asthma drug) compared with the inhalation route which was found to be free from side effects⁴. Studies have shown that the amount of salbutamol found in the blood is higher after intravenous administration than after inhalation treatment^{5,6}. With this in mind, the results obtained on the effects of a drug administered intravenously may be misleading if the drug is intended for inhalation.

The intention was to assess the effect of the drug on blood pressure and heart activity. However, the time required for the cardiovascular system of a conscious cynomolgus monkey to recover from excitement induced by handling stress is at least 30 minutes⁷ (even after intravenous injection with a saline solution). In this experiment, blood pressure and heart activity measurements were taken from the monkeys 30 minutes after injection, and so it would be difficult to separate the effects of the drug on the heart from the effects of stress due to handling. "*Because monkeys are promptly excited under experimental procedures, it is often difficult to evaluate drug effects on the cardiovascular system*".

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Furthermore, testing potential asthma drugs on animals is an unscientific and clumsy way to advance medical science. It has been acknowledged that animal 'models' of this disease are not able to exactly reproduce the pathology of human asthma⁸, and it has been said that "An animal model can never be the perfect match of a human disease, if only because it occurs in another species and is deliberately induced rather than developing spontaneously"⁹, and further, that: "...there are no reliable animal models of asthma and short-term human challenge studies do not accurately reflect long-term anti-inflammatory activity, so companies should press ahead with clinical trials in patients as soon as possible..... Pharmacokinetic studies in healthy volunteers are also an essential part of the drug development process and should be conducted as early as possible"¹⁰.



Modern solutions

Asthma drugs fall into two main types – relievers and preventers. There are already a variety of drugs readily available on the market in both categories. Relievers are used in treating asthma attacks and in preventing exercise-induced bronchospasm. The prevention drugs are anti-inflammatory; they are usually taken every day to control the problem. In addition to these two main types of asthma medicines, there are others which can be used in combination with both types if needed.

However, asthma treatment has not depended upon animal experiments. Sodium cromoglycate (intranasal) has been used in the treatment of asthma since 1968. The khellin derivative, which is responsible for the protective effect of the substance, was discovered by Roger Ernest Collingwood Altounyan, a doctor who had little faith in animal experiments. He was allergic to guinea pigs so he exposed himself to them to induce asthma attacks, against which he tested over 600 new drugs.

Nevertheless, there is a range of non-animal alternative methods – advanced techniques – which can be employed for the development and testing of asthma drugs and this is the way forward:-

- ECITTS (Integrated Toxicity Test Scheme). *In vitro* tests have been developed to assess the speed and routes taken by toxins through the body and their effects on individual tissues and organs¹¹
- Human Tissue Samples – Lung Derived Fibroblasts
Inflammatory conditions can be mimicked *in vitro* for the study of gene expression regulation (a key factor in asthma). Lung derived fibroblasts (cells in connective tissue that synthesise collagen) extracted from human lung tissue, obtained during the surgical removal of bronchial tumours. Fibroblasts can be cultured and treated with the protein involved in asthma, and treated with a drug; then analysed using polymerase chain reaction and ELISA respectively.
- *In vitro* Lung Model: Investigates mechanisms behind occupational asthma. Isocyanates under test are dissolved in phosphate buffered saline to mimic the physiological conditions of the lung. (Isocyanates are widely used in industry in the production of paints and polyurethanes, and are one of the most common inducers of occupational asthma). The immune cell responses to isocyanate is investigated using a cell line.

However, more drugs is not necessarily the only answer with asthma. It is known that asthma is caused by a combination of environmental and genetic factors². Considerable evidence has been produced to suggest that incidences of asthma are increasing and that this is as a consequence of environment and lifestyle changes. Therefore the root causes of this asthma "epidemic" need to be addressed. The primary prevention objective of the World Health Organisation's Chronic Respiratory Diseases Programme is to reduce the level of exposure of individuals and populations to common risk factors, particularly tobacco, poor nutrition, frequent lower respiratory infections during childhood and environmental air pollution³.

1 Detweiler DK (1993), as cited in conference reports. ATLA 21: 106-107

2 Finkelstein JN and Johnston CJ (2004) Enhanced sensitivity of the postnatal lung to environmental insults and oxidant stress. *Pediatrics* 113 (4): 1092-1096

3 http://www.drugs.com/PDR/Pulmicort_Turbuhaler_Inhalation_Powder.html

4 Sharma RK et al. (1985) FEV1 changes following oral, inhalational and intravenous salbutamol administration in patients with bronchial asthma. *Lung India* 3 (4): 177-181

5 Janson C (1991) Plasma levels and effects of salbutamol after inhaled or IV administration in stable asthma. *European Respiratory Journal* 4: 544-550

6 High-dose inhaled versus intravenous salbutamol combined with theophylline in severe acute asthma. *European Respiratory Journal* 1990; 3: 163-170

7 Hassimoto M and Harada T (2003) Use of a telemetry system to examine recovery of the cardiovascular system after excitement induced by handling stress in a conscious cynomolgus monkey (*Macaca fascicularis*). *Journal of Medical Primatology* 32 (6): 346-352

8 Isenberg-Felig H, Justice JP and Keane-Myers A (2003) Animal models of allergic asthma. *Current Allergy and Asthma Reports*

9 Furness PN and Harris K (1994). University of Leicester. *International Journal of Experimental Pathology* 75: 9-22

10 *Scrip* (1994) Volume 1919 p. 23.

11 Balls M. et al (2000) *Progress in the Reduction Refinement and Replacement of Animal Experiments*. Elsevier, Amsterdam, pp525-536

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Project 658952: rats die being forced to breathe paint

Ten Sprague Dawley rats were immobilised in tubes and then forced to inhale an aerosol spray of anti-fouling paint for three hours, for Hempel's Marine Paints, of Denmark. Inveresk described the probable severity of this procedure as 'substantial'. It was clear the rats were going to experience severe suffering and die a horrible, agonising death.

The liquid paint and air was passed through an electric spray gun, and then into an exposure chamber at a controlled concentration, filling the chamber with aerosolised red paint. Each animal was then loaded into a tapered restraint tube which fitted into the exposure chamber, so that only their noses were exposed.

The rats were observed for reaction, and clinical signs recorded at 30 minute intervals. However, over 50% of the animals died during dosing, so the procedure was terminated after 3 hours. The survivors were found to be at the point of death, so were killed. The suffering of these animals was appalling; from approximately 30 minutes into the test their breathing had become slow and laboured – yet their suffering was allowed to continue for a further 2.5 hours. The survivors were cold to the touch with pale extremities and were comatose.

Three males and three females were found dead 1-3 hours after dosing commenced. The remaining four animals were killed afterwards. Therefore, the mortality rate as a result of exposure to the paint was 100%.

The post mortem examination showed that 7 out of the 10 rats had reddened lungs. The only conclusion that could be drawn from the experiment was that the paint showed significant toxicity. A value for the lethal concentration which would result in the death of 50% of the animals (LC50) could not be established. No other conclusions could be drawn.

Yet this was scheduled as a non-lethal 'Limit Test' (where theoretically if no ill-effects occur at a pre-selected maximum dose, no greater quantities are given). Limit Tests were introduced as a more humane alternative to the LC50 (Lethal Concentration 50%) test, where the aim was to kill 50% of the animals in order to discover the lethal amount – Inveresk's experimenters managed to undertake a Limit Test which greatly exceeded the brutality of the old test, with 100% mortality.

It is hard to imagine the suffering of these rats, trapped and utterly immobile, with the only air available thick with choking paint – steadily filling their noses, throats and lungs. And for what? An unnecessary, inconclusive experiment for yet another tin of paint.

Typically in acute inhalation toxicity studies, groups of 10 animals are tested at differing dose concentrations and dose concentrations are tested sequentially. Where extreme toxicity is not expected from known data, the maximum concentration may be tested first. In this study, all 10 rats were exposed to the same concentration of paint for the same period of time. This concentration caused 100% mortality in the animals under test. There is no indication in the report whether it was indeed the maximum concentration which was tested in this study. If there was no literature or data which suggested that deaths should not occur as a result of inhalation of this single concentration of the paint, then the methodology of this approach is questionable. Lower concentrations should have been tested first.

Where deaths do occur, additional testing at lower concentrations is normally required. Because no meaningful conclusions could be drawn from the results of this study, it may mean that more rats were exposed to the aerosolised paint in order to obtain the information required.

Critical Review

Differences between species make this an entirely inappropriate and ill-thought out test. Unlike humans, rats are obligatory nose breathers and an inhaled substance will react with the first site of contact, i.e. the nasal passage. In humans, the mouth will also be the target for an inhaled substance^{1,2}. There is also good evidence to suggest that lung size, shape and biochemistry affect inhalation toxicology results³. Obviously the structure and chemical makeup of a rat's lung is different to that of humans. Resistance to toxicity has even been shown to differ between rats and mice⁴, which are closely related, therefore any attempt to apply the results of tests on rats to the human situation introduces further uncertainty and misleading conclusions.

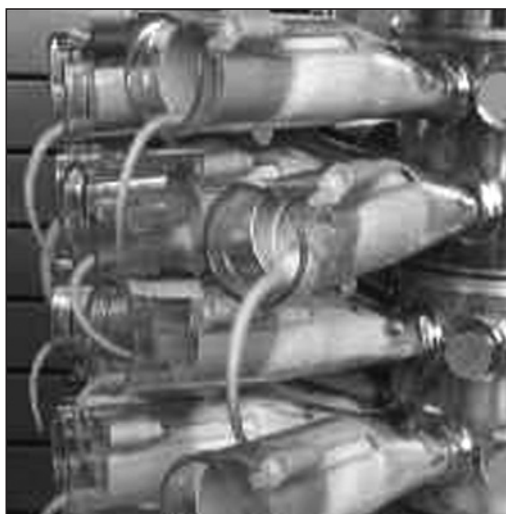
It is well known that restraint can be highly stressful to mammals and it may also lead to suppression of the immune system⁵. It is therefore highly probable that the stress experienced by the rats in the restraining tubes could have affected experimental results, because the tolerance to toxic substances would have been reduced.

There is also evidence to suggest that lung size, shape, and biochemistry affect inhalation toxicology results³, and that the stress caused to rats in such an experiment can affect the enzyme activity in their lungs, affecting the results. Such tests have

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been criticised: "...uncertainties limit the usefulness of the rat inhalation bioassay for quantitative purposes. For example, in the conclusion of their draft report, the Office of Environmental Health Hazard Assessment decided to base their recommended unit risk on human epidemiology instead of the rat bioassay because 'the uncertainty in the scaling of rat predictions to humans is substantial'. Similarly, the Clean Air Scientific Advisory Committee has recommended to the Environmental Protection Agency that the rat inhalation bioassay, conducted under overload conditions, is "not relevant for human risk assessment"².

The aim was to investigate the inhalation toxicity of the paint when used by humans. Anti-fouling paint is used on the underside of boats, so normal human exposure would be when the paint is applied. This test was therefore a waste of resources and animals lives for several reasons: firstly, regulations are in place in Europe to properly protect workers and the work will be conducted wearing masks and in a well-ventilated area^{6,7}. Secondly, there is information about the toxicity of various types of paint already available. Thirdly, it is highly unlikely that humans would be exposed in a situation where the atmosphere is composed entirely of aerosolised paint, and not for a continuous 3 hour period.



Immobilising the rats in the restraint tubes could have affected results. For example, it has been shown that immobilisation stress in rats can affect enzyme activity within the lungs⁸. Since the lung response is crucial to assess acute inhalation toxicity, this could make a significant difference to the results.

Exercise during exposure to inhaled toxicants increases inhaled dose rate and alters dose distribution within the respiratory tract, and exercise has long been recognised as a critical exposure variable⁹. However, while modern inhalation exposure studies with human subjects routinely use an exercise protocol, most investigations with animal models are performed with resting subjects (including this one). Animal inhalation exposures under exercising conditions frequently induce respiratory toxic effects well beyond that expected from the simple increase in ventilation dose rate⁹. Due to the fact that the rats were not only resting but physically restrained in tubes during exposure, the results from this experiment are likely to be highly misleading.

It is important to note that the results of several chronic inhalation experiments have suggested that rats may be more prone than other rodent species to develop persistent tumours and cell enlargements in response to the accumulation of inhaled particles. In addition, rats and primates differ in their lung anatomy and rate of particle clearance from the lung. Rats and mice clear particles from the lung relatively quickly, whereas monkeys and humans clear particles more slowly. The extensive anatomical differences between the faster-clearing and slower-clearing species could be responsible for this. Therefore, the responses to inhalation experiments in rats may not be predictive of responses in humans who inhale particles at concentrations representing high occupational exposures¹⁰.

Consideration should also be given to the question of whether data from rats exposed to high concentrations of particles (which greatly exceed expected human exposure concentrations) should be used to quantitatively predict toxicity effects in humans exposed at lower rates¹⁰.

Modern solutions

Anti-fouling paints are used to coat the bottom of sea vessels in order to prevent barnacles and molluscs (in fresh and salt water) from growing on the hull. Unless this growth is prevented, the ships can be slowed down and fuel consumption is increased. GLOBIC SP-ECO 81900 is one of 7 of anti-fouling paints currently manufactured by Hempel. At least three of these (81900 included) contain the same active ingredients. In addition to Hempel's own products, there are also many other similar products already on the market. There is insufficient need for any further paints to be marketed, especially if this process requires the use of animals for toxicity testing. Anti-fouling ships paints are currently causing environmental concern as they dissolve slowly into water and can cause pollution^{11,12,13}.

There are alternatives to the use of animals in such tests. In addition to information already available on computer databases, *in vitro* techniques such as CULTEX can be introduced. CULTEX measures airborne substances in relation to human health. The target cells are exposed directly at the air/liquid interface and the exposure device is connected to aerosol-generating systems. Dose-dependent cytotoxic effects are then measured in human lung epithelial cells which are exposed to air contaminated with single gases or complex mixtures, such as diesel exhaust fumes and cigarette smoke.

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Using Human Tissue Samples¹ obtained during facial surgery, can create an *in vitro* model that mimics the structure and function of cells found in the mouth. This has been proven to predict inhaled formaldehyde toxicity and could be developed for use with other substances.

¹ Hedberg JJ et al. (2001) ATLA 29: 325-333

² Valberg PA et al. (1996) Regulatory Toxicology and Pharmacology 24: 30-44

³ Foster JR et al. (1985) British Journal of Experimental Pathology 66: 203

⁴ Cunningham ML (2002) The mouse is not a rat is not a human: Species differences exist. Toxicological Sciences 70: 157-158

⁵ Poole T (1997) Laboratory Animals 31: 116-124

⁶ Health and Safety Executive Information Document HSE 730/15: Safe use of tin-free, marine anti-fouling coatings (http://www.hse.gov.uk/fod/infodocs/730_15id.pdf)

⁷ National Paint and Coatings Association: Certification training for safe handling and use of anti-fouling paints containing tributyltin (TBT) (<http://pested.osu.edu/documents/Boat%20Antifouling%20Paint.pdf>)

⁸ Kovacheva S and Ribarov S: Pulmonary xanthine oxidase activity of rats exposed to prolonged immobilisation stress (<http://www.gpb.sav.sk/gbp498.htm>)

⁹ Mautz WJ (2003) Exercising animal models in inhalation toxicology: interactions with ozone and formaldehyde. Environ Res 92 (1): 14-26

¹⁰ Nikula KJ et al. (1997) Sites of particle retention and lung tissue responses to chronically inhaled diesel exhaust and coal dust in rats and cynomolgus monkeys. Environmental Health Perspectives 105 (Supplement 5): 1231-1234

¹¹ Kennish MJ (1998) Pollution Impacts on Marine Biotic Communities. CRC Press, New York.

¹² Ranke J (2002) Persistence of antifouling agents in the marine biosphere. Environmental Sciences and Technology 36: 1539-1545

¹³ Katranitsas A, Castritsi-Catharios J and Persoone G (2003) The effects of a copper-based antifouling paint on mortality and enzymatic activity of a non-target marine organism. Marine Pollution Bulletin 44: 149-155

Project 574899: rabbits die for breast implants

Hutchinson International, Inc. of the USA commissioned Inveresk to see whether breast implant materials can cause irritation in rabbits. In an experiment described by Inveresk as likely to cause 'moderate' suffering, the backs of 3 rabbits (supplied by Harlan UK) were shaved, and six pieces of a breast implant device (BIOJCH), together with plastic tubing and a catheter, were inserted into the muscles in their backs. There were eight implant sites per rabbit.

The breast implant device consisted of 3 components: a valve (via which the filler material is inserted), a seal and an outer envelope (which holds the filler material). As the incisions healed and the immune system attacked the foreign bodies sewn into their muscles, it is likely that the rabbits would have experienced significant pain and discomfort.

The animals were then observed daily for 4 days post-implantation and at least twice weekly thereafter for any reactions. The body weight of each animal was recorded twice weekly. All 3 animals were killed after 28 days.

No clinical signs of toxicity were noted, but the rabbits suffered redness around the implant sites, loss of weight, one rabbit had large scratches on its back, another had bruising, and some implant sites on the third rabbit remained open, including one where the skin was unable to move over the muscle. The post mortem examination found severe thickening of the skin, chronic inflammation, chronic congestion, haemorrhaging and deterioration and death of tissue. New fibrous tissue had formed as a result of the inflammation. Some implants could not be found as they had migrated. The conclusion reached was that "BIOJCH was well tolerated," and any reactions were "to be expected when a foreign body is implanted."

Critical Review

The Inveresk report states that: "Under the conditions of the study, there was a mild chronic or chronic active inflammatory response at each test site due to intramuscular implantation of BIOJCH. This response is expected with implantation of a foreign body, and the reaction was milder than that elicited by the positive control implant. The inflammatory response was more severe to the sutures than the implanted materials."

However, according to the tissue and post-mortem findings, the claim that the reaction to BIOJCH implantation was milder than the positive control is inaccurate. Post mortem results show a marked to severe thickening of the skin (fibrosis), chronic inflammation, chronic congestion, haemorrhaging (including some severe cases) and the deterioration and death of tissue around implants at the BIOJCH sites. At the positive control implantation sites, there was minimal to moderate thickening of the skin, some deterioration and death of tissue around implant sites, and some inflammation.

Due to some of the materials migrating around the animals' bodies, it was not possible to properly assess the reaction to all of the materials and thus properly finalise the study.

The Inveresk experiment not only caused much pain and suffering, but also failed scientifically: Firstly, it did not satisfy guidelines for this type of procedure as the implants had migrated around the animals' bodies and therefore the local effects on their tissues at the original implant sites could not be assessed.

Secondly, it was stated that the route of application of the test material was selected because it has been defined as the route of human exposure. However the breast implants were sewn into the muscles around the rabbits' spines. Also, the animals' muscle fibres were separated and then the muscle, connective tissue and skin layers were stitched back together separately. In humans, breast implants are not implanted intramuscularly, and are implanted either between the breast and the chest muscle or behind the chest muscle⁵.

The results obtained from this experiment cannot be directly applied to humans – not only because of the structural and physiological differences between humans and rabbits, but because both the anatomical position of the implants and their intramuscular insertion do not replicate what would happen in the human situation.

Moreover, there are 40 years of human experience of breast implant materials and a wealth of data. Indeed, breast implants are now discussed in terms of to which 'generation' they belong. Breast implant materials have also been tested on animals previously – many times over. Human data could have been used.

Studies of breast implant materials in rats have shown that the rate of degradation in the rat is much faster than in humans, indicating that rat studies cannot be used to draw conclusions regarding biodegradation in humans¹. A study to assess the potential adverse effects of the outer envelope of a silicone breast implant on general reproduction and fetal development in rats and rabbits attempted to make its results relevant to humans by using a size of implant which was approximate to the expected body burden of women with breast implants². It could not however overcome the overriding problem that neither a rat nor a rabbit in any way resembles a human female. In one study, 18 out of a total of 21 rabbits implanted with silicone shell implants on either side of the chest wall survived more than 3 months³.

Although breast implant materials have been tested in laboratory animals since the early 1950s, a standardised evaluation system does not exist in which human breast implant materials are exposed directly to the mammary area of female animals⁶. Evaluating the biological performance of a material depends on the unique biological characteristics of the anatomic site of implantation⁷. Human-based methods need to be used to test materials that are intended for application in humans as biomaterials, medical devices or prostheses⁸. All such materials undergo tissue responses when implanted into living tissue. These responses include injury, inflammatory and wound healing responses, foreign body reactions, and the fibrous encapsulation of the material⁴.

Modern solutions

BIOJCH was tested as a breast implant device with a valve through which filler such as saline or silicone is inserted. Implants such as these are used because they enable the surgeon to increase or decrease the amount of filler in the implant post operatively. Silicone gel breast implants have been used since the beginning of the 1960s⁹, and saline-filled breast implants have been used in breast augmentation surgery since the late 1960s and early 1970s¹⁰. In addition, there are already a variety of "inflatable implants" available with a variety of different valves for filling¹¹. All of these implant devices are made from silicone; only the fillings differ¹². As mentioned earlier, there is therefore a wealth of relevant human data already available regarding the toxicity and short and long terms effects of different breast implant devices.

Thus, the study on rabbits for BIOJCH was completely unnecessary, for the following reasons:-

- there is already a wealth of data available on similar implantation devices, in humans
- it was repetition, similar materials had already been tested:
In the 1960s, the US National Institutes of Health implanted a series of materials in animals to see how living tissue responded. The objective was to see if these synthetic materials could be used in breast implant devices. It was concluded that a range of synthetic materials including nylon, Teflon, Mylar, polyester, silicone and polyethylene could be implanted in animals over a long period of time with minimal irritation or other physical response¹³.
- breast augmentation is mostly cosmetic. Breast augmentation is in the majority of instances an elective and cosmetic procedure, and even with animal test results, it carries a number of health risks including: bleeding, infection, hardening of the breast due to scar tissue shrinkage, rupture, scarring, change in nipple sensation and skin wrinkling¹². Breast implants can also hinder the detection of breast cancer as they can make mammography more difficult. For medical reconstructive surgery, there are plenty of safe materials on the market.

If further development of breast implant materials is required, the emphasis should be on using the wealth of human data already available, and *in vitro* techniques, for example breast tissue engineering involves using fat tissue based on a patient's own cells to rebuild the breast after a mastectomy.

1 Sinclair TM, Kerrigan CL and Sampalis J (1995) Biodegradation of polyurethane foam revisited in the rat model. *Plastic and Reconstructive Surgery* 96 (6): 1326-1335
2 Siddiqui et al. (1994) Reproductive and developmental toxicity studies of silicone elastomer Q7-2423 Q7-2551 in rats and rabbits. *Fundamental and Applied Toxicology* 23 (3): 377-381
3 Young VL et al. (1991) Biocompatibility of radiolucent breast implants. *Plastic and Reconstructive Surgery* 88 (3): 462-474
4 International Organisation for Standardisation (<http://www.iso.org/iso/en/CatalogueDetailPage.CatalogueDetail?CSNUMBER=18966&scopelist>)
5 NHS Direct Online Health Encyclopaedia: Breast Implants (<http://www.nhsdirect.nhs.uk/en.asp?TopicID=721&AreaID=4725>)
6 Devor DE et al. (1993) Development of an animal model for testing human breast implant materials. *Toxicologic Pathology* 21 (3): 261-273
7 Clinical Applications of Biomaterials. NIH Consensus Statement Online 1982 Nov 1-3; 4 (5): 1-19
8 Anderson J (2001) Biological responses to materials. *Annual Review of Materials Research* 31: 81-110
9 <http://www.medical-devices.gov.uk>
10 http://www.breast-implants-augmentations.com/silicone_breast_implants.htm
11 <http://www.justbreastimplants.com>
12 Department of Health. Breast Implants: Information for women considering breast implants.
13 <http://www.chemcases.com/silicon/si115one.htm>

Animal Experiments at Inveresk

Code of Practice for the Housing and Care of Laboratory Animals

Conditions of environment, housing facilities, and care of laboratory animals under the Animals' (Scientific Procedures) Act 1986 is provided for in the *Code of Practice for the Housing and Care of Animals Used in Scientific Procedures* (COP), published by the Home Office. These are the 'strict guidelines' so often cited by defenders of animal experimentation. Inveresk itself states: "*The Home Office have published a Code of Practice for the housing and care of animals used in research, with which we must comply. In fact in many areas Inveresk exceeds the minimum requirements of the Code of Practice.*" In reality this code offers poor protection to laboratory animals as for most species there is little detail, and the code can be ignored with apparent impunity.

Investigations of the use of animals in laboratories in a dozen establishments over the past decade by the National Anti-Vivisection Society and Animal Defenders International have concluded that for most species the COP offers very little in terms of detailed requirements for animal comfort or environmental enrichment, and for those where there is some detail (such as dogs and primates), this is routinely ignored.

The adverse effect of conditions of housing and husbandry on animals at Inveresk are noted in their own reports. There were several incidences of fighting reported in the studies where dogs were housed in groups, with on occasion more than half of the animals in one study sustaining fighting injuries.

COP 2.8 deals with staff competence in correct animal handling and restraint and in the procedures which they will be carrying out. There were several errors by personnel during the experiments we have examined: A monkey was unable to use her leg "*considered to be due to an injury, possibly caused during study procedures on day 1 of dosing*". During an anti-inflammatory drug test a dog became decannulated twice and had to be removed and replaced with another dog; two dogs in one test were accidentally dosed into the lungs instead of the stomach. In another test for Finrozole, a urinary dysfunction drug, another dog was lung dosed.

Several times in the COP the need for the environment to meet the animals' needs is stressed – we have identified the relevant paragraphs from the COP:-

COP 2.3. "*The 1986 European Convention (20) provides that:... Any restriction on the extent to which an animal can satisfy its psychological and ethological needs shall be limited as far as practicable...*"

COP 2.10 Notes that animal house design should provide a suitable environment, including any special requirement for exercise or social contact for the species to be housed. COP 3.2 reiterates: "The aim is to maintain animals in good health and physical condition; behaving in a manner normal for the species and strain and with a reasonably full expression of their behavioural repertoire..."

COP 3.21 states "*Size, shape and fittings of pens and cages should be designed to meet the physiological and behavioural needs of the animals. The shape of the cage and the furniture provided may be as important to the animal as the overall size of the cage. Social relationships are as important as stocking densities and room must be allowed for growth of the animals...*"

The dogs at Inveresk appear to live in small barren pens with a scattering sawdust/woodchips. This is likely to be one of the reasons for the high incidence of fighting. These are typical laboratory standards and have also been observed in the UK at SmithKline Beecham, Toxicol Laboratories, Charing Cross and Westminster Medical School, and Huntingdon Life Sciences. The dogs' meagre space at Inveresk is supplemented by 30 minutes of exercise a week – few would consider this sufficient to keep a pet dog healthy and mentally stimulated.

The macaque monkey cages at Inveresk are also very limited in space, although there is a small concession in that some cages are linked to allow some additional movement and socialisation. Nonetheless the photographs we have indicate no serious attempt at environmental enrichment or meeting the "*physiological and behavioural needs of the animals*".

COP 3.25 states: "*Animals should be housed so that they can be easily inspected: animals should not be held in cages which are stacked so high that they cannot be inspected without removing them from the rack*".

Rodent rooms at Inveresk, as we have seen at other laboratories, are filled with racks of cages. We do not believe it is possible to adequately check these animals without removing them from their racks. Any individual care and attention for these animals is made almost impossible by the sheer number of animals – Inveresk boasts experimenting on approximately 25,000 animals per year. There are additional problems with inspection because of protective clothing worn in animal rooms.

COP 3.28 states: "*Bedding and nesting material should be provided, unless it is clearly inappropriate. It should be comfortable for the particular species...*"

We have never observed dogs provided with bedding material in a UK laboratory, other than a thin scattering of

Animal Experiments at Inveresk

sawdust/woodchips to soak up urine which is clearly to aid cleaning and not comfort for the inhabitants. Neither primates nor dogs at Inveresk appear to have any bedding that could be described as comfortable.

COP 3.11 acknowledges the stress of transport and notes that *"Time in transit should be kept to a minimum"*. Yet for one study dogs were brought all the way from the USA.

The COP gives more guidance on primates than almost any other species, yet we have shown these were routinely ignored during our studies at St Mary's Hospital Medical School and the Institute of Neurology. There is little indication of compliance at Inveresk.

COP 3.43 notes: *"Primates have high intelligence, most have arboreal habits and all need complex, stimulating environments"*.

COP 3.45 states: *"Housing should be secure and present the minimum hazard to the handlers and to the animals. Within this constraint, it should provide adequate space, complexity (e.g. varied diets, cage furniture) and opportunities for social interaction. Food items such as nuts, grain etc. may with benefit be added to bedding to encourage foraging behaviour and reduce boredom"*.

COP 3.54 states: *"Cages should include wooden perches, a swing, a wooden nest box, a shelf for feeding and sufficient wood shavings to allow foraging. The animals should have space to jump horizontally from one perch to another"*.

The macaque monkeys shown in the photographs at Inveresk have no bedding material and therefore nothing in which to forage; there is no complexity in their environment, just minimal cage furniture. Such facilities would not be deemed acceptable in a zoo, yet some laboratory animals (including primates) might spend a year (or more) in these cages; for example animals kept for breeding or those in long-term studies.





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