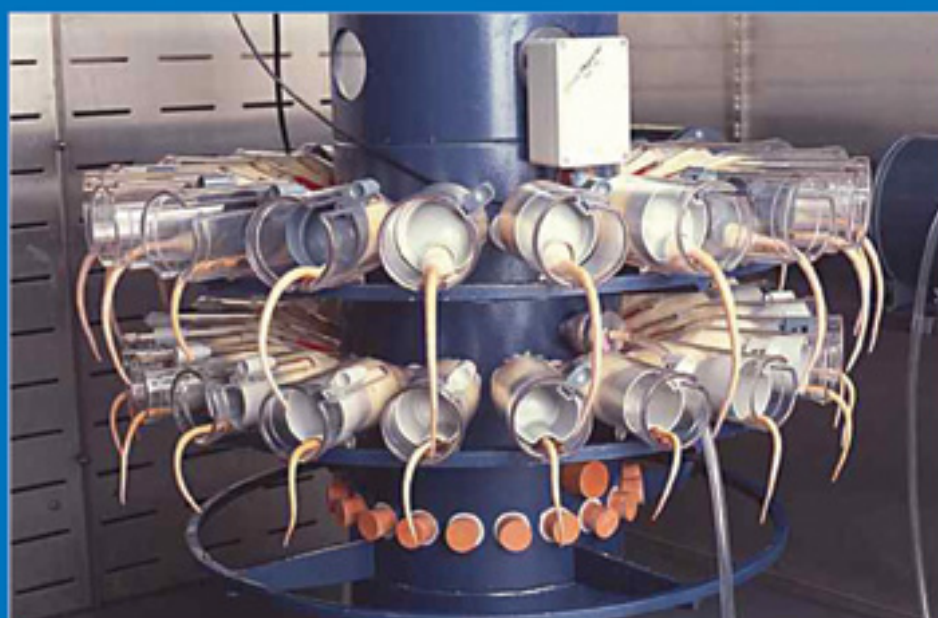


Keep animals out of REACH



Acute Toxicity Testing



**Animal Defenders International
National Anti-Vivisection Society
Lord Dowding Fund for Humane Research**

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**KEEP ANIMALS OUT OF REACH:
Supplementary Report
ACUTE TOXICITY TESTS**

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INTRODUCTION

This briefing has been produced by Animal Defenders International (ADI), the National Anti-Vivisection Society (NAVS), and the Lord Dowding Fund (LDF), for the UK Government, the European Commission and Members of the European Parliament.

Here, we present compelling evidence to demonstrate that the acute toxicity tests in the REACH proposal should adopt a non-animal approach.

Animal Defenders International is putting forward evidence against the adoption of an animal acute toxicity test for Annex V on the grounds that such tests have in the past proved wholly inconclusive, and caused severe animal suffering.

This is in light of the imminent vote (4th October 2005) by the EU Environment Committee on the amendments proposing to add an animal test for acute toxicity to Annex V of REACH, and the one from Caroline Lucas, MEP, proposing a non-animal testing strategy. ADI understands that amendments adopted by the Environment Committee on this date will then go forward for the Plenary vote in November.

There is a worrying possibility that one of the animal test additions might be adopted. This means that at the Plenary Vote, the only option for MEPs will be to either vote for an acute toxicity animal test for Annex V, or to leave the proposal in its current state, without any acute toxicity data being sought for the low volume chemicals.

As the evidence in this report indicates, the adoption of an acute toxicity animal test for Annex V, and indeed the use of animal tests throughout the entire REACH proposal, could have huge implications for human safety. Using animal tests to obtain safety data for acute toxicity, and other chemical safety data, is unreliable, unethical and unnecessary.

The evidence we present here, details of an **acute toxicity test on animals**, is based on leaked photographs and documents from **Inveresk Research International**, a contract testing laboratory based near Edinburgh in Scotland. 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 a horrific report of an acute toxicity test undertaken in rats to obtain safety data for an anti-fouling paint, and the protocol which refers to it.

We discuss the conduct of the experiment, the suffering of the animals, its justification, relevance to the human situation, and the existing data which was available at the time. We analyse the experiment in the context of the Annex V proposals, and explain why it should never have been allowed to take place. We discuss that this experiment, and others like it, are **unreliable, unethical and unnecessary**. The need to stringently apply the information found in Annex IX, which indicates under which conditions testing should be waived, is subsequently highlighted by our evidence. This is in order to not only prevent unnecessary animal suffering, but also to ensure that only the more relevant human data determines the safety information for chemicals.

The evidence detailed here has serious implications for members of both the European Parliament and UK Government who are now considering the REACH chemical regulation proposals, and possible amendments to it. It has been 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, the UK Government and MEPs must seriously consider whether Europe even has the research capacity to undertake the animal testing currently demanded within REACH.

Moreover, they must consider whether we really want contract testing laboratories such as Inveresk obtaining chemical safety data, when there are obviously very serious inadequacies in the methods by which they obtain it.

Our recommendations for a more effective and humane testing strategy under REACH have already been circulated to the European Commission, UK Government, MEPs and UK 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 supplement to our earlier report hopes to more fully inform parliamentarians about the real meaning of REACH – the day-to-day reality for animals.

SUMMARY

Companies such as Inveresk will undertake the animal tests detailed in the EU's REACH chemicals regulations, including the acute toxicity tests which, it is being proposed, should be added to Annex V.

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..."*

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.

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. Before they vote on the inclusion of an animal test for acute toxicity in Annex V, they also need to question the possibly dire implications, for both humans and animals, of doing so, in light of the evidence presented here.

BACKGROUND

On 29 October 2003, the European Commission adopted a proposal for a new EU regulatory framework for chemicals. REACH (Registration, Evaluation and Authorisation of Chemicals) will regulate the manufacture, import and use of chemical substances, and create a new European Chemicals Agency.

Under the proposed REACH system, enterprises that manufacture or import more than one tonne of a chemical substance per year would be required to register it in a central database. The aims of the new Regulation are the protection of human health and the environment while maintaining the competitiveness and enhancing the innovative capability of the EU chemicals industry, as well as the avoidance of use of animals in testing. REACH would furthermore give greater responsibility to industry to manage the risks from chemicals and to provide safety information on the substances. This information would be passed down the chain of production.

ANIMAL TESTING

In order to obtain the required safety information on these chemical substances, millions of animals will be used. The aims of REACH are to protect human health and the environment, but are also likely to bring about the painful deaths of these millions of animals, in experiments that are unreliable, unethical, and unnecessary. Additional animal tests are now being proposed for inclusion, including those for acute toxicity.

We all want to see dangerous chemicals removed from our environment, but REACH will cost billions of euros and the lives of millions of animals – and the animal tests involved will not deliver safe chemicals for people or the environment, especially those for acute toxicity.

The current REACH animal testing programme has been criticised by experts as being cumbersome, expensive, and unlikely to achieve its aims (Source: Royal Commission on Environmental Pollution). For example, it took 11 years to produce risk assessments for 140 chemicals, yet REACH proposes that industry produce similar data for 30,000 chemicals, in the same timescale. The Strategic Partnership on REACH Testing (SPORT), initiated by The European Chemical Industry Council (CEFIC) has also concluded that REACH in its present format will not be workable without the significant simplification of concepts and requirements, and that there should be guidance on how the use of existing data can be maximised¹.

TESTING INVOLVED IN THE REACH PROPOSAL

Annexes V to VIII of the REACH proposal include lists of tests which will be involved in the chemical safety testing programme.

- **Annex V** includes standard information requirements for substances manufactured or imported in quantities of 1 tonne or more. In order to meet this requirement, data will generally be collected with non-animal tests, with Annex V listing *in vitro* skin and eye irritation, *in vitro* skin corrosivity, *in vitro* mutagenicity, and non-animal ecotoxicity. There is one animal test listed, the Local Lymph Node Assay for the determination of skin sensitisation. There is also an additional test proposed for acute toxicity; the vote will determine whether this will be an animal or non-animal test.
- **Annexes VI, VII and VIII** provide additional standard information requirements for substances manufactured or imported in quantities of 10, 100, and 1,000 tonnes or more respectively. They are mainly based on animal tests and do not include all validated non-animal tests currently available.
- **Annex X** (previously Annex V of the Dangerous Substances Directive (67/548/EC), which provides a list of test methods, does not include all *in vitro* and other non-animal tests currently available; Annex X details the animal test protocols.

TOXICOLOGICAL AND ECOTOXICOLOGICAL TESTS IN THE REACH ANNEXES

Annex V – all chemicals

- Skin irritation or skin corrosion (*in vitro*)
- Eye irritation (*in vitro*)
- Skin sensitisation (Local Lymph Node Assay)
- Mutagenicity (*in vitro*)
- Proposal to include an acute toxicity test (*in vivo* or *in vitro*)

Annex VI – above 10 tonnes

- Skin irritation (*in vivo*)
- Eye irritation (*in vivo*)
- Mutagenicity (*in vitro*)
- Acute toxicity (*in vivo* by oral route and/or by inhalation and/or dermal route)
- Repeated dose toxicity (28-day *in vivo* study, one species, male and female)

- Reproductive toxicity (Screening for developmental toxicity *in vivo*, if there is no (Q)SAR estimates that the substance may be a developmental toxicant. A positive result in the screening should be confirmed by further *in vivo* tests)
- Toxicokinetics – if it can be derived from the available information
- Short term toxicity testing on fish
- Adsorption/desorption screening

Annex VII – above 100 tonnes

- Mutagenicity *in vivo* – if positive results from the Annex V and VI tests
- 28-day repeated dose toxicity or sub-chronic toxicity study (90-day, one species, rodent)
- Developmental toxicity study (two species)
- Two-generation reproductive toxicity study (*in vivo*, one species, male and female)
- Long term toxicity testing on fish (fish early-life stage toxicity test or fish short term toxicity test on embryo and sac-fry stages or fish juvenile growth test)
- Accumulation in one aquatic species, preferably fish
- Further studies on adsorption/desorption depending on results of studies required in Annex VI

Annex VIII – above 1,000 tonnes

- If appropriate, further mutagenicity studies shall be proposed
- A long term (12 month) repeated toxicity study may be proposed
- Two generation reproductive toxicity study, one species, male and female, if not provided as part of Annex VII requirements
- A carcinogenicity study may be proposed by the applicant or requested by the competent authority
- Long term or reproductive toxicity in birds

ANNEX IX: GENERAL RULES FOR ADAPTATION OF THE STANDARD TESTING REGIME SET OUT IN ANNEXES V TO VIII

The standard testing regime for all substances may be adapted for the following accepted reasons:

1. TESTING DOES NOT APPEAR SCIENTIFICALLY NECESSARY due to:

- **Existing data** (physical-chemical properties, animal experiments, historical human data). Data shall be considered to be equivalent to data generated by the corresponding test in Annex X if certain conditions are met; e.g. if exposure duration is comparable, and if adequate and reliable documentation is provided.
- **Weight of evidence** from several independent sources (including newly developed test methods) indicating that a substance has/has not a particular dangerous property. Where sufficient weight of evidence for the presence or absence of a particular dangerous property is available:
 - further testing on vertebrate animals for that property shall be omitted
 - further testing not involving vertebrate animals may be omitted.
- **Structure-activity relationship (SAR)**. Results obtained from valid qualitative or quantitative structure-activity relationship models ((Q)SARs) may indicate the presence or absence of a certain dangerous property.
- ***In vitro* methods**. Results obtained from suitable *in vitro* methods may indicate the presence of a certain dangerous property. In this context, “suitable” means sufficiently well developed according to internationally agreed test development criteria (e.g. the ECVAM* criteria for the entry of a test into the prevalidation process). Depending on the potential risk, immediate confirmation requiring testing beyond the information foreseen in Annex V or VI or proposed confirmation requiring testing beyond the information foreseen in Annex VII or VIII for the respective tonnage level may be necessary. If the results obtained from the use of such *in vitro* methods do not indicate a certain dangerous property, the relevant test shall nevertheless be carried out at the appropriate tonnage level to confirm the negative result, unless testing is not required in accordance with Annexes V to VIII or the other rules in Annex IX. (*European Centre for the Validation of Alternative Methods).

Such confirmation may be waived, if certain conditions are met:

- **Grouping of substances and read-across approach.** Substances whose physicochemical, toxicological and ecotoxicological properties are likely to be similar or follow a regular pattern as a result of structural similarity may be considered as a group, or "category" of substances. Application of the group concept requires that physicochemical properties, human health effects and environmental effects or environmental fate may be predicted from data for a reference substance within the group by interpolation to other substances in the group (read-across approach). This avoids the need to test every substance for every endpoint. The similarities may be based on:
 - 1) a common functional group
 - 2) the common precursors and/or the likelihood of common breakdown products via physical and biological processes, which result in structurally similar chemicals, or
 - 3) a constant pattern in the changing of the potency of the properties across the category.

2. TESTING IS TECHNICALLY NOT POSSIBLE

Testing for a specific endpoint may be omitted, if it is technically not possible to conduct the study as a consequence of the properties of the substance: e.g. very volatile, highly reactive or unstable substances cannot be used, mixing of the substance with water may cause danger of fire or explosion or the radio-labelling of the substance required in certain studies may not be possible.

3. SUBSTANCE-TAILORED EXPOSURE-DRIVEN TESTING

Testing in accordance with Annexes VII and VIII may be omitted, based on the exposure scenario(s) developed in the Chemical Safety Report. The implementation of substance-tailored exposure-driven testing would ensure that new testing is only undertaken when novel data are required to make a meaningful and necessary risk assessment.

ACUTE TOXICITY TESTS

On 4th October 2005, the EU Environment Committee will vote on the possible, and final, amendments to REACH in accordance with the conditions found in Annex IX. Annex IX sets out general rules for adaptation to the testing regime set out in Annexes V to VIII; therefore this annex indicates the conditions under which certain tests can be waived.

In Annex V, there is currently no provision for acute toxicity tests and the European Council of Ministers would like one to be included. The Alliance of Liberals and Democrats for Europe (ALDE) and the Green Party would also like to see an acute toxicity test incorporated into Annex V, but will support *in vitro* techniques in preference to animal studies. It is possible that validated alternatives to acute toxicity tests on animals for classification and labelling of products would probably be accepted.

Therefore, as part of their vote, the Environment Committee will be voting on the proposal to add an animal test for acute toxicity to Annex V of REACH, and the proposal put forward by Caroline Lucas, MEP, proposing a non-animal testing strategy for this. The amendments which are successfully voted in and adopted by the Environment Committee will then go forward for the plenary vote, which is fully attended by all qualified members.

Animal Defenders International is putting forward evidence against the adoption of an animal acute toxicity test for Annex V on the grounds that such tests have in the past proved wholly inconclusive, and caused severe animal suffering.

HISTORY OF THE ACUTE TOXICITY TEST

Historically, lethality has been the primary toxicological endpoint in acute toxicity tests, which have been in use for nearly 80 years. For the majority of this time, *in vivo* (animal) tests have been used. The acute toxicity test in rodents is seen and used by many as a defining step in achieving the toxicity of a test material

for the purpose of hazard classification and labelling. It is designed to determine adverse effects and to estimate the dose level that is expected to kill 50% of the test population (i.e., the LD50).

Trevan (1927) was the first to attempt to standardize a method for assessing the toxicity of potent biological toxicants, the progenitor of the "lethal dose, 50% (LD50) test"¹⁵. The classical LD50 test procedure that evolved from this innovation in the 1970s and early 1980s used from 100 to 200 animals per test substance¹⁶. Although other information, such as the slope of the dose-response curve, confidence interval for the LD50, and toxic signs, could also be obtained from this test, the procedure was severely criticized for both scientific and animal welfare reasons. It was claimed that the 'for the recognition of the symptomatology of acute poisoning in man, and for the determination of the human lethal dose, the LD50 is of very little value'¹⁷. These criticisms eventually resulted in the proposal and adoption of a new guideline (OECD TG 401; OECD, 1987) that reduced the required number of animals to 20. This became the most widely used method for defining the acute toxicity of a chemical and a mandatory-testing requirement for new chemicals.

More recently, the acute toxicity test procedure has been modified in various ways to refine and further reduce the number of animals used to a maximum of 16 (OECD, 1992; 1996; 1998b). For testing acute toxicity by the oral route, the LD50 method has been replaced in the OECD guidelines by other protocols which use fewer animals, such as the Fixed Dose Procedure, Up and Down Procedure, and the Acute Toxic Class Method, which all serve to provide an estimated LD50 value.

For testing acute toxicity by inhalation or by the dermal (skin) route, LD50/LC50 tests are still most predominantly used. By looking at the history of the acute toxicity test, this indicates that in spite all of the scientific advances made in the last 80 years, modern researchers are still using a method for obtaining acute toxicity data that has been criticised since its inception, and which has had to be altered due to the extent of animal suffering that it causes.

As already established, the use of acute toxicity data is mainly to label and classify chemicals based on their toxicity, for application to the human situation. However, there are a number of intrinsic problems associated with doing this.

RELEVANCE OF ANIMAL TESTS TO THE HUMAN SITUATION

- Human exposures to the chemicals tested are most frequently at a low-dose, repetitive level rather than by a single massive dose, which is how animals are exposed to the test substances.
- There is variation in the way different species deal with and react to chemicals. These differences can include the rates and routes of metabolism and in absorption, distribution, and excretion; the target organs involved; and sensitivity to toxicity. This is why existing data on acute toxicity in humans should take always priority over data obtained from animal tests, and should be found and used wherever possible.
- There is often a high degree of variability in acute toxicity data obtained from rodents of different ages, sexes, and genetic strains. Weight, diet, temperature, humidity and other environmentally dependent variables can also cause a great deal of discrepancy between results, and can account for why the results obtained from different laboratories can differ so widely.
- Relying on animal tests is costly and time-consuming, and can therefore have negative implications since relying on animal tests will delay the timely regulation which human health protection demands.
- Animal tests for acute toxicity have never been validated to modern standards, and their reproducibility between laboratories is so poor that if a validation study were to be carried out, acute toxicity tests would fail.

THE EVIDENCE:

SUMMARY OF THE INVERESK EXPERIMENT

Inveresk Project Number 658952:

Exposure of an anti-fouling paint (GLOBIC SP- ECO 81900) to rats by snout-only nasal inhalation

Subject:	Acute inhalation toxicity of an anti-fouling paint in rats
Report Number:	18239
Title:	Antifouling GLOBIC SP-ECO 81900 Acute Inhalation Toxicity Study in Rats (Limit Test)
Animals Used:	10 Sprague Dawley rats (5 Male and 5 Female): 8-10 weeks old; 176-251g
Test Material:	Anti-fouling paint
Administration Method:	Nasal inhalation
Type of Study:	Acute inhalation toxicity (limit test)
Experimental Start Date:	18 th October 1999
Duration:	3 hours
Date of Completion:	18 th October 1999
Research Location(s):	Room N66, Block N Rodent Inhalation Toxicology Complex, Elphinstone Research Centre, Inveresk Research, Tranent, EH33 2NE, Scotland.
Sponsor(s):	Hempel's Marine Paints A/S, Lundtoftevej 150, DK-2800 Lyngby, Denmark.
Animal Supplier(s):	Charles River (U.K.) Limited, Manston Road, Margate, Kent, England (10 animals)
Objective of Study:	To investigate the acute inhalation toxicity of aerosolised Hempel's Antifouling GLOBIC SP-ECO 81900 in rats following a single 3-hour snout only exposure
"Probable Severity Limit":	"Substantial": this is the severity limit assigned by Inveresk to the experimental protocol(s) which apply to this experiment

Summary

- 10 Sprague Dawley rats were subjected to a test atmosphere containing aerosolised Hempel's Antifouling GLOBIC SP-ECO 81900 paint. They were exposed to the aerosolised paint via snout-only inhalation for a single 3-hour period.

- The test atmosphere was generated by passing liquid paint and air through an electric spray gun. The aerosolised paint was then passed into an exposure chamber at a controlled concentration, filling the exposure chamber with aerosolised red paint.
- The animals were restrained inside transparent tubes: Each animal was loaded into the tapered restraint tubes, which fitted into the exposure chamber. The animals' snouts were at the narrow end of the tube and thus were the only parts of the rats exposed to the test atmosphere. The rest of the animal's body was contained in the wider portion of the tube which extended outwards from the exposure chamber. All 10 animals were loaded into the tubes and exposed to the test atmosphere at the same time.
- All the rats were observed continuously throughout the exposure period for signs of reaction to exposure, and any clinical signs were noted at 30 minute intervals. Due to the fact that over 50% of animals were found dead during dosing, the dosing procedure was terminated 3 hours after dosing commenced.
- The surviving animals were removed from the chamber, unloaded from the restraint tubes, returned to their cages and observed. The animals that survived dosing were found to be at the point of death, and so were all killed humanely post-dosing. No body weights were taken after dosing as all the animals had died or had been humanely killed. All animals were subjected to a post-mortem examination.

Mortality:

- 3 males and 3 females were **found dead between 1 to 3 hours** after dosing commenced. The remaining 4 animals were killed post-dose. Therefore, the **mortality rate** as a result of exposure to the paint was **100%**.

Clinical Signs:

- **Slow and laboured respiration was seen from approximately 30 minutes** into the exposure period in all animals.
- Red staining on the rats' heads and snouts were seen in all animals during the exposure period.
- Immediately post-dosing, **slow and laboured respiration continued and the surviving animals were cold to touch, showed pale extremities and were comatose**. They were therefore humanely killed.

Post-mortem Examination: 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.**

UNRELIABLE

Species Differences

Known 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^{2,3}. There is also good evidence to suggest that lung size, shape and biochemistry affect inhalation toxicology results⁴. 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 mice⁵, 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 system⁶. 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.

Tests such as this have 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.

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.

Furthermore, 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 exposures¹¹. 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 rates¹¹.

Relevance to Human Situation

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:

- regulations are in place in Europe to properly protect workers and the work would be conducted wearing masks and in a well-ventilated area^{7,8};
- there is information about the toxicity of various types of paint already available;
- 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 lungs⁹. Since the lung response is crucial to assess acute inhalation toxicity, this could make a significant difference to the results.

Experimental Procedure

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 variable¹⁰. 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 rate¹⁰. 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.

In the Inveresk report, there is reference to the use of a disinfectant solution of Tego 2000, supplied by Goldschmidt and Company Limited, Middlesex, to wash the walls and ceiling of the animal room. Some Tego products are quite toxic, and some animals are very vulnerable to some disinfectants. The potential

toxicity of the disinfectant does not appear to have been taken into account. This would seem an odd omission, as the food and water were analysed thoroughly to avoid possible effects on results.

In the report, it states that the concentration of paint used 'greatly exceeded the 5.0 mg.litre⁻¹ required by the protocol.' This appears at odds with the (leaked) protocol which states: "The upper limit required to be tested (the limit dose) is normally 2000mg/litre." There is no mention of 5.0 mg.litre⁻¹.

Shortly thereafter it is stated: "Since it was not possible to assess the active biocide separately, the complete formulation was evaluated". No reason is given as to why such assessment was not possible.

In Annex I (sections 0.1 and 0.2) of the REACH proposal, it states:

*"The purpose of this Annex is to set out how manufacturers and importers are to assess and document that the risks arising from the substance they manufacture or import are adequately controlled during manufacture and their own use(s) and that others further down the supply chain can adequately control the risks. The chemical safety assessment shall address all the identified uses. It shall consider the use of the substance on its own (including any major impurities and additives), in a preparation or in an article. The assessment shall consider all stages of the lifecycle of the substance as defined by the identified uses. The chemical safety assessment shall be based on a comparison of the potential adverse effects of a substance with the known or reasonably foreseeable exposure of man and/or the environment to that substance."*²⁰

In Annex Ia (section 11) of the REACH proposal, it states:

*"This section deals with the need for a concise but complete and comprehensible description of the various toxicological (health) effects, which can arise if the user comes into contact with the substance or preparation. The information shall include dangerous-to-health effects from exposure to the substance or preparation, based on, for example, test data and experience. The information shall also include, where appropriate, delayed, immediate and chronic effects from short- and long-term exposure: for example sensitisation, narcosis, carcinogenicity, mutagenicity and reproductive toxicity (developmental toxicity and fertility). It shall also include information on the different routes of exposure (inhalation, ingestion, skin and eye contact), and describe the symptoms related to the physical, chemical and toxicological characteristics. Taking account of the information already provided under heading 3, composition/information on ingredients, it may be necessary to make reference to specific health effects of certain substances in the preparation."*²¹

In Annex Ib (sections 1 and 2) of the REACH proposal, it states:

"A chemical safety assessment for a preparation shall be conducted in accordance with Annex I with the following modifications:

1. INFORMATION BASE

The chemical safety assessment for a preparation shall be based on the information on the individual substances in the preparation contained in the technical dossier and/or the information communicated by the supplier in the safety data sheet. It shall also be based on the information available on the preparation itself.

2. HAZARD ASSESSMENTS

The hazard assessments (human health, human health for physicochemical properties and environmental) shall be carried out in accordance with Sections 1, 2 and 3 with the following alterations:

- (a) *For the evaluation of data step(s), any relevant data for the preparation, the classification for each substance in the preparation and any specific concentration limits for each substance in the preparation shall be presented.*
- (b) *For the classification and labelling step, the classification and labelling for the preparation in accordance with European Parliament and Council Directive 1999/45/EC shall be presented and justified."*²²

However, the chemical safety assessment for the paint undertaken via this acute toxicity test in rats only considered the final preparation of the paint. It did not consider the substances constituting the paint, and could therefore be considered to not have fully provided the safety information for the product in question (GLOBIC SP-ECO 81900). It did not make reference to the specific health effects of certain

substances in the preparation, nor did it take into account specific concentration limits for each substance in the preparation. If it had done, then **the substantial and prolonged suffering of these animals could have been avoided.**

Discrepancies in Inveresk's Experimental Report

There appears to be a major inconsistency in dates given in the report. On page 5 the date of the Quality Assurance Inspection and report of this to management is 5 November 1998. Yet the study initiation date given on page 9 is 2 November 1999. Further possible errors are the apparent inconsistency of the experimental start and completion dates being earlier than the study initiation date.

The protocol states that exposure periods for acute toxicity inhalation tests are normally one hour or less. The reason for the 3 hour time period for this study is therefore questionable.

There is an extraordinarily high level of apparent inconsistency and error in the report. These include discrepancies in figures relating to total aerosol concentrations, LC₅₀ values, mass median aerodynamic diameter (MMAD) values, and values for the % of solid-phase aerosol mass < 4.2 µm.

Some of these errors are substantial and may, if carried into the final report, have led to major errors being transferred to material safety data sheets (MSDSs) and therefore have potential implications for human safety. There is some odd usage of symbols, with a colon apparently being frequently used instead of the µ symbol representing the prefix 'micro-'. Although this may appear minor, it relates to a crucial value (particle size), and it is possible that these unorthodox symbols contributed to some numerical errors.

In the report it states: "*Reddened lungs were noted in all lobes in 2 male and 5 female animals. Otherwise no adverse effects were detected*". This appears misleading. Presumably it means that no other adverse effects were noted *in the lungs*.

We find it curious that there were no other effects noted in the lungs, bearing in mind the laboured breathing and the deaths. Other possible changes might include those indicating hypoxia (oxygen starvation) and stress injuries to blood vessels. The term 'reddening' appears vague, and it is not clear whether the reddening was due to the colour of the paint or stress-induced damage involving damage to tissue, bleeding, etc.

Inveresk's report also states that *all* organs were examined post-mortem. Inhaled substances are absorbed into the bloodstream and travel around the body, as do substances ingested, injected and absorbed through the skin, yet no effects are noted in the necropsy findings other than the reddening of the lungs. No cause of death is given for the rats which died during the experiment, an omission we find unacceptable.

Furthermore, there are no indications as to which rats died during the experiment and which were euthanised. So the report does not provide information on whether the rats which had reddened lungs and various lung and body weights died during the experiment, or were euthanised.

Another apparent anomaly is that, according to the report, all animals showed staining of the test compound on both snout *and* head. The experimental procedure is stated in the protocol and in the report, it says that the experimental equipment is designed to expose only the nose/snout. This may indicate that the apparatus used was not fit for the purpose for which it was used, and that this would result in inaccurate measurements of limits or LC₅₀s, as some product failed to reach the nose. (It might, however, be absorbed through the skin, which would also confound the results, as these should only represent *inhalation* toxicity.)

Summary:

Not only was the experiment designed and conducted in such a way that it failed to provide the data which it was intended to provide, but the results were also written up so carelessly that any potentially useful information was not recorded, and multiple numerical errors mean that incorrect data may be transferred to safety data sheets and other health-and-safety-related materials, with potential implications for human safety.

UNETHICAL

Animal Suffering

Slow and laboured respiration was seen from approximately 30 minutes into the exposure period in all animals. However the exposure period continued for further 2.5 hours, during which time slow and laboured respiration in the rats continued.

There are references to the 'animal room' and to the 'animal holding room'. It is not clear whether these describe the same room. In the report, it is stated that the inhalation procedure was conducted in a laboratory adjacent to the animals' holding room. It is also stated *that "surviving animals were removed from the chamber, unloaded from the restraint tubes, returned to their cages in the animal holding room and observed for clinical signs...the animals that survived dosing...were killed immediately post dosing"*.

This appears contradictory; it does not sound as though the killing was at all immediate. Surely it would have been apparent that these rats were at the point of death as soon as they were removed from the restraint tubes. They should have been humanely killed at that point, rather than being transferred back into their cages to be observed in a comatose state.

The mortality rate as a result of exposure to the paint was 100%. However the dosing procedure was only terminated 3 hours after dosing commenced "due to the fact that over 50% of animals were found dead during dosing." Had this not occurred, the rats may have been forced to inhale the paint for a longer period of time, whilst still suffering from slow and laboured respiration (the typical exposure period for acute inhalation toxicity studies is 4 hours).

This experiment 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 however managed to undertake a Limit Test which greatly exceeded the brutality of the old test, with 100% mortality.

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 later exposed to the aerosolised paint in order to obtain the information required.

UK and European Legislation

Council Directive 86/609/EEC (1986)

Article 5(e):

"Member States shall ensure that, as far as the general care and accommodation of animals is concerned, arrangements are made to ensure that any defect or suffering discovered is eliminated as quickly as possible."

However, although the rats were seen to be at the point of death when they were removed from the experimental apparatus, they were not killed immediately. Rather they were transferred back into the holding room before they were euthanized.

Article 7.2:

“An experiment shall not be performed if another scientifically satisfactory method of obtaining the result sought, not entailing the use of an animal, is reasonably and practicably available.”

As will become clear in the following, the LC₅₀ for the paint, sought through the rat inhalation test conducted in this experiment, could have been amassed from LC₅₀ values which were already available.

1998 Amendments to the Animals (Scientific Procedures) Act 1986

Section 5; subsection (5)

“The Secretary of State shall not grant a project licence unless he is satisfied –

- a) that the purpose of the programme to be specified in the licence cannot be achieved satisfactorily by any other reasonably practicable method not entailing the use of protected animals; and*
- b) that the regulated procedures to be used are those which use the minimum number of animals, involve animals with the lowest degree of neurophysiological sensitivity, cause the least pain, suffering, distress or lasting harm, and are most likely to produce satisfactory results.”*

Not only did this experiment cause substantial and prolonged suffering to the animals involved, the information the experiment sought to obtain was already available at the time.

UNNECESSARY

In this section we present the evidence which shows that the Inveresk rat experiment was not justified, both in terms of animal suffering and that existing data on the paint's ingredients (individually and in preparations) were already available at the time when the experiment was undertaken.

This evidence also indicates why this rat test would have been waived under Annex IX of the REACH proposals, and therefore why the **unnecessary animal suffering** seen in this experiment should never have been allowed to take place.

This case highlights how important it is that the chemicals industry and contract testing laboratories involved in the testing for REACH, stringently apply the criteria set out in Annex IX to avoid the unnecessary use of animals, **and that legislation enforces this**. It also highlights the importance of using sophisticated and advanced scientific methods to obtain more relevant data.

Annex IX

Annex IX indicates the conditions under which testing should be waived under the REACH proposal. These conditions stipulate that further testing should not be undertaken **if testing does not appear scientifically necessary**, due to the presence of (amongst others):

- **Existing data**
- **Weight of evidence**

The availability of both of these and their application to the situation (as explained below) should have indicated that there was no need for the Inveresk rat test to be undertaken, especially when it involved such a high paint concentration. If similar animal acute toxicity tests to this are undertaken in the REACH proposals, and adopted as an amendment to Annex V, then the legitimacy of the manufacturers and contract testing laboratories undertaking such tests could be debated. This is especially in light of the fact that, despite the extensive availability of pre-existing data, the manufacturers and researchers at Inveresk still allowed the horrific rat test presented in this report to take place.

Ingredients of GLOBIC SP- ECO 81900

The likely composition of GLOBIC SP- ECO 81900 has been ascertained from a number of data searches. The manufacturers of the paint (Hempel) are reluctant to publish full ingredients lists for commercial reasons. Therefore, national government sites, patent applications, downloaded documents, and sites relating to similar products, have all been used to collate the necessary information on the likely ingredients of the paint and its percentage composition²⁵⁻³⁸.

The exact ingredients making up approximately 80% or more of the paint are as follows:

- **Cuprous Oxide** (36.1-38.2%)
- **Zinc Resinate** (estimated 30-40%)
- **Xylene** (about 13.5%)
- **Sea Nine** (1.86% or 1.9%).

The zinc resinate (a.k.a. zinc carboxylate) is part of a binder system made with synthetic rosin (possibly 35-50% by volume of solid content of the paint, the resinate making up possibly over 60% by volume of solid content of the binder).

The other ingredients are (possibly):

- **Zinc Oxide** in place of some of the copper oxide
- **Petroleum Distillates** (possibly up to 5% by weight of paint) – possibly xylene
- (possibly) **Bentonite as filler** (possibly 4-5% by weight and/or 4 parts by volume of solid content of paint)
- (possibly) **Polymeric Flexibilizers** (possibly 2-15% by volume of solid content of the paint) in the binder system
- **Fibres** (probably of a biodegradable mineral type) (probably about 5% by volume of solid content of the paint)
- (possibly) **Dyes and Additives** (0-15% by volume of solid content of the paint, (possibly) 6% for additives (N.B. **Bentonite** is also listed as an additive in a patent application), additives also possibly including a product called Lanco MIKAL 00180 or similar, apparently an **antistat or wax additive**, at (possibly) 4-8% by volume of solid content of paint; and (possibly) **titanium dioxide** at (possibly) 4-5% by volume of solid content of paint.

Evidence of Acute Inhalation Studies Undertaken Before 1999

(a) On similar complete products

A 'similar complete product' as defined here refers to a paint which contains the same major ingredients as GLOBIC SP-ECO 81900, i.e. **cuprous oxide, zinc resinate and xylene**, which together are likely to make up 80% or more of the total weight of the paint.

- An Australian paint known as Antifouling Seavictor 50, produced by Jotun Paints/Jotun Marine Coatings, appears particularly close to GLOBIC SP- ECO 81900. It contains cuprous oxide, zinc oxide and xylene²³. The Material Safety Data Sheet (MSDS) for this product does not contain any LC₅₀ values, but does carry warnings regarding its inhalation toxicity:
 - *“Vapour is irritating to mucous membranes and respiratory tract. Can cause dizziness, headaches, nausea and may lead to unconsciousness. Prolonged exposure to vapour may cause damage to the central nervous system.”*
 - Although the final version of this data sheet was prepared on 13th June 2000, it is highly likely that this information would have been known earlier, i.e. prior to the initiation of the rat acute toxicity inhalation study conducted at Inveresk.
- A whole-product rat inhalation LC₅₀ for Devoe ABC No. 3 Black AF is 8000 ppm 4hr (8 mg per litre over 4 hours). This product is a marine antifouling paint.

The MSDS for this product gives the following information³⁹:

“Effects of Exposure: BREATHING: IRRITATION OF RESPIRATORY TRACT; HEADACHE, NAUSEA, DIZZINESS, WEAKNESS, FATIGUE. EXTREME EXPOSURE: UNCONSCIOUSNESS, RESPIRATORY ARREST. SKIN/EYE: IRRITATION. HARMFUL IF ABSORBED THROUGH SKIN. INGEST: STOMACH/INTESTINAL IRRITATION, NAUSEA, VOMITING, DIARRHOEA. PERMANENT BRAIN & NERVOUS SYSTEM DAMAGE. DUST DISEASE OF LUNGS”.

This MSDA is dated 09/26/1990 (US usage, thus 26 September 1990) with a review date of 12/09/1998 (9 December 1998). The xylene content of this paint is 9.7% (cf. 13.5% in ‘Hempel’s Antifouling Globic’) and the cuprous oxide content is 47.1% (substantially higher than for Globic paints).

Annex Ib (section 2) of the REACH proposal, states that:

*“For the derivation of derived no-effect levels (DNELs), the DNEL for each substance in the preparation with an appropriate reference to the safety data sheet of the supplier shall be listed, as well as the DNEL derived for the preparation, with a justification on their derivation. **In lack of any information to the contrary, then additivity of effects shall be assumed.** The DNELs for the preparation can then be calculated for each route of exposure and each exposure scenario as a weighted average of the DNELs for each substance in the preparation, with the weights being the fraction of the exposure to the substance in the preparation to the total exposure to all substances in the preparation.*

*For the derivation of the predicted no-effect concentrations (PNECs), the PNEC for each substance in the preparation with an appropriate reference to the safety data sheet of the supplier shall be listed, as well as the PNECs derived for the preparation, with a justification on their derivation. **In lack of any information to the contrary, then additivity of effects shall be assumed.** The PNECs for the preparation can then be calculated for each environmental sphere and each exposure scenario as a weighted average of the PNECs for each substance in the preparation, with the weights being the fraction of the exposure to the substance in the preparation to the total exposure to all substances in the preparation.”* (our emphasis)

Although this relates to DNELs and PNECs, it would seem logical to also use this approach to LC₅₀s where the LC₅₀ for a whole preparation is not known. We have therefore taken this approach with regard to cuprous oxide and xylene.

(b) On ingredients:

(i) Cuprous Oxide

From a list of suppliers of cuprous oxide⁴¹, we have obtained information which includes the Safety Description S22 and wording ‘Do not inhale dust’. Cuprous oxide has been used as a biocide for a long time, so is clearly known to be toxic.

From an International Uniform Chemical Information Database (IUCLID) data sheet for cuprous oxide, the following information is obtained:

- Rat inhalation LC₅₀s are given as ‘ca. 5 mg/l’ for the company Colorificio Attiva Genoa (no date given), ‘ca. 5 mg/l’ for Nordox Industrier A/S Oslo test in 1985 and >50 mg/l for Norddeutsche Affinerie Hamburg in 1988. No references are given for these experiments.
- 5mg/l or thereabouts appears to be the predominant LC₅₀ value given for cuprous oxide from other sources viewed, suggesting that the higher value >50 mg/l reflects experimental error.
- Taking the middle value for the concentration of the paint used in the Inveresk report: 20.70 mg/litre and multiplying it by 38.2% - the proportion by weight of cuprous oxide in ‘Hempel’s Antifouling Globic’ as described²⁵ (and assuming that its specific gravity/density is the same as that for Globic SP-ECO 81900) - we get a concentration of cuprous oxide in the test atmosphere of
20.70 mg/l x 38.2%
= 7.91 mg/l.

Thus the lethality of the paint used in the rat experiment could have been entirely due to the cuprous oxide.

This is especially clear when one takes into account the facts that 60%, rather than 50%, of the rats died within three rather than four hours (the normal exposure period for such tests), and that some appear to have died early in the experiment.

Had Inveresk taken this data into account before the rat experiment was undertaken, then it would have been clear that this acute toxicity test would never achieve the required results. This is because the paint concentration used would have been lethal based on the amount of cuprous oxide in the paint alone, and the experiment would therefore be inconclusive.

(ii) Zinc Resinate

The analysis of several data sheets gives the same Permissible Exposure Limit (PEL) for zinc resinate. This is 0.05 mg/litre of zinc.

Permissible Exposure Limit is a time-weighted average (TWA) or absolute value (usually prescribed by regulation) setting out the maximum permitted exposure to a hazardous chemical.

The American Conference of Governmental and Industrial Hygienists' threshold limit value (ACGIH TLV) for zinc resinate is also given as 0.05 mg/litre of zinc. This is the value expressed as a time-weighted average; the concentration of a substance to which most workers can be exposed without adverse effects.

Several Material Data Safety Sheets, including one from the USA⁴², list zinc resinate as a *'toxic chemical subject to the reporting requirements of Section 313 of Title III of the Emergency Planning and Community Right-To-Know Act of 1986 and 40 CFR Part 372'*.

Calcium zinc resinate, which has CAS no. 68334-35-0, is also listed under: *'TOXIC CHEMICAL OR CHEMICALS SUBJECT TO THE REPORTING REQUIREMENTS OF SECTION 313 OF TITLE III OF THE EMERGENCY PLANNING AND COMMUNITY RIGHT-TO-KNOW ACT OF 1986 AND 40 CFR PART 372'*.⁴³

Zinc resinsates containing calcium have been used in Hempel paints⁴⁴.

Therefore we can conclude that the data available on zinc resinate shows that it is a toxic chemical, and thus paints containing it as an ingredient would also be toxic.

This information, which indicated the risk that zinc resinate posed to human health, would already have been available before the Inveresk rat test was undertaken.

(iii) Xylene

From an International Uniform Chemical Information Database (IUCLID) data sheet for xylene, it becomes clear that a number of rat inhalation tests have already been conducted on the chemical⁴⁵⁻⁴⁸. All of these were conducted many years before the Inveresk rat test was undertaken, some as many as 20 years previously.

From these documents and Material Safety Data Sheets - excluding those which appear to be highly erroneous (by orders of magnitude) - the rat inhalation LC₅₀ for xylene appears to be between 4.55 mg/litre and 6.35 mg/litre.

Taking an LC₅₀ value for xylene midway between those found and referred to above, excluding the apparently-erroneous one, we get
(4.55 + 6.35)/2 mg/l
= 5.45 mg/l.

Taking the middle value for the concentration of the paint used in the Inveresk report: 20.70 mg/litre and multiplying it by 13.5% - the proportion by weight of xylene in 'Hempel's Antifouling Globic' as described²⁵ (and assuming that its specific gravity/density is the same as that for Globic SP-ECO 81900) - we get a concentration of xylene in the test atmosphere of $20.70 \text{ mg/l} \times 13.5\% = 2.79 \text{ mg/l}$.

Thus $2.79/5.45 = 51\%$ of the lethality seen in the Inveresk experiment could have been due to the xylene alone.

Most LC₅₀ values are for 4-hour exposures.

Whilst it may not be valid to assume that there is a linear relationship between exposure time and lethality, or between test atmosphere concentration and lethality, it is perhaps helpful to make some rough calculations based on such assumptions:-

Six rats (60% of the total) died rather than 50%, so perhaps the LC₅₀ could be assumed to be $50/60 \times 20.70 = <17.25 \text{ mg/l}$.

Furthermore, some rats died early in the experiment, exposure was for three rather than four hours, and the surviving rats were so ill that they had to be euthanised.

So one might assume that 100% of the rats would have died after four hours exposure, and therefore that the LC₅₀ of the paint might actually be $50/100 \times 20.70 = <10.35 \text{ mg/l}$.

This is approaching the range found for xylene, further supporting a theory that much, most or perhaps even all the lethality could have been caused by xylene.

Xylene also causes laboured breathing, a symptom observed early in the Inveresk test.

The ChemIDPlus record for xylene⁴⁹, as well as giving a rat inhalation LC₅₀ from 1974 as 5000 ppm/4H, i.e. 5 mg/litre, also gives lowest published toxic concentration (TCLo) for inhalation in humans in a 1943 report as 200 ppm, i.e. 0.2 mg/l, and a human LCLo from the British Medical Journal in 1970 as 10 000 ppm, i.e. 10 mg/l.

LCLo is defined variously online as:

- lowest lethal airborne concentration tested;
- the lowest concentration of a material in air reported to have caused the death of animals or humans. The exposure may be acute or chronic. This is also called the lowest concentration causing death, lowest detected lethal concentration, and lethal concentration low; *and*
- lowest concentration at which death occurred

In March 1998, it was stated that: *"Human and animal data show that all xylene isomers or xylene mixtures produce similar effects, although specific isomers may not be equally potent in producing the effects. Acute (short-term) inhalation exposure to mixed xylenes in humans has been associated with dyspnea and irritation of the nose and throat; gastrointestinal effects such as nausea, vomiting, and gastric discomfort; mild transient eye irritation; and neurological effects such as impaired short-term memory, impaired reaction time, performance decrements in numerical ability, and alterations in equilibrium and body balance... (US EPA)."*... inhalation rat LC₅₀: 5000 ppm/4H

In 2003, it was also stated that *"Pulmonary effects have been documented in occupational exposures to undetermined concentrations of mixed xylenes (and other solvents) and include **laboured breathing** and impaired pulmonary function...Animal data are consistent with human data in documenting respiratory effects from xylene exposure. Acute and subacute exposures in mice, rats, and guinea pigs have been associated with; **decreased respiratory rate; laboured breathing; irritation of the respiratory tract; pulmonary edema; and pulmonary inflammation** by a number of studies."*⁵¹

Although this document is dated May 2003, it cites much older data, including the following:

1. "Exposure to concentrations of 2,440 ppm mixed xylene for **6 minutes** (Korsak et al. 1988), to 1,467 ppm o-xylene for 5 minutes (De Ceaurriz et al. 1981), or to 1,361 ppm m-xylene for 6 minutes (Korsak et al. 1993) produced a **50% decrease in respiratory rate in mice**. Comparison of the individual xylene isomers showed that the irritant effects of m- and o-xylene as quantified by measurements of respiratory rate in mice are more pronounced than those of p-xylene, with o-xylene having the most prolonged effect (Korsak et al. 1990)."
2. "**Acute Exposure in Humans:** After exposure to about 700 ppm xylene (calculated) for up to one hour, headache, nausea, irritation of the eyes, nose and throat, dizziness, vertigo and vomiting have been reported (Klaucke et al., 1982)."
3. "In mice (Swiss-Webster) exposed to 1300 ppm xylene for **one minute**, a **decrease in respiratory rate** as an indication of **respiratory tract irritation** was seen (Carpenter et al., 1975). This effect was not seen at an exposure level of 460 ppm."
4. "On the basis of human volunteer studies (Anshelm Olson et al. 1985), one may conclude that the **NOAEL (no observed adverse effect level) for acute CNS (central nervous system) effects in humans is about 304 mg/m (70 ppm) for a 4hr exposure.**

To convert ppm to mg/litre, it is necessary to divide by 1000. Thus the values causing serious adverse effects are substantially lower than the concentration of xylene used in the Inveresk experiment, which was 2,790 ppm.

Even if the correct concentration of paint - 5 mg/l - had been used (as stated in the protocol), the concentration of xylene would still have been 675 ppm, just below the level reported above to cause serious adverse effects in humans after an hour or less, well above the lowest published toxic concentration (TCLo) for inhalation in humans in a 1943 report, and possibly high enough to cause the reported effects in mice and depression of the human CNS.

(iv) Petroleum Distillates

Petroleum Distillates are a **possible ingredient** for the GLOBIC SP-ECO 81900 paint, which was tested on the rats in the Inveresk experiment. One of the petroleum distillates in the paint could be **Naphtha**.

A document which is published by the Consumer Product Safety Commission, Washington, DC⁵², states that petroleum distillate compounds can cause **similar toxic effects to those of xylene**. The document also states: "*The toxicity of petroleum distillates and other hydrocarbons affects the respiratory system. Aspiration of small amounts of these chemicals directly into the lung...can cause **chemical pneumonia, pulmonary damage, and death.***"

The ChemIDPlus record for naphtha⁵³ gives its inhalation LCLo as 30000 mg/kg (human) and 1600 mg/kg (rat), from a study conducted in 1939.

These values have been converted to those values used elsewhere in this report:

1.23 kg/m³ is the density of air⁵⁴. 1 cubic metre = 1 000 litres.
Thus a kg of air contains 1/1.23 m³ or 1 000/1.23 litres = 813 litres.

So the human LCLo for naphtha is 30 000 mg/813 litres = 36.9 mg/litre.

and the rat LCLo for naphtha is 1 600 mg/813 litres = 1.96 mg/litre.

Thus data available long before the Inveresk rat test was conducted, gave lethal toxicity information for petroleum distillates in both humans and rats.

In conclusion, the Inveresk report refers to a protocol which recommends the use of a paint concentration of 5 mg/litre. This is the concentration reported in several documents to be the LC₅₀ for two principal ingredients (xylene and cuprous oxide), but Inveresk used a concentration more than four times this value.

This raises two questions:

- Why did they not follow the protocol?
- Does the recommendation to use this concentration reflect the fact that there is already a high degree of certainty as to the LC₅₀ of such products? If so, this would be **further evidence that the tests may have been unnecessary under sub-section 1.1.2 (1), (2) and (4) of Annex IX of REACH.**

Dangerous Preparation Directive (1999/45/EC)

The objective of the Dangerous Preparation Directive (DPD) is similar to that of the Dangerous Substances Directive (DSD), but relates to preparations and finished products.

The DPD's prime aim is to identify and control dangerous finished product formulations.

The DPD can communicate safety properties of finished product formulations by prescribing specific classification and labelling measures for preparations. In addition it can prescribe certain packaging measures. Whether and how any of these measures is applied depends on the safety properties of the finished product. Products not classified as dangerous will not be subject to any labelling or packaging measures.

The DPD also describes the methodology to assess the safety properties of finished products. This methodology integrates - through a calculation method - safety properties of individual ingredients as defined by the Dangerous Substances Directive. In this, the "hazard" approach of the DSD, which does not take exposure into account, is transferred to the DPD as well.

Apart from this integration approach, the DPD also acknowledges the use of certain animal tests and human experience data to reach a particular conclusion on which measures to take.

The manufacturer is required to carry out the classification, labelling and packaging of his finished products as indicated in the Dangerous Preparations Directive and provide a Material Safety Data Sheet to the professional user of the product. The authorities monitor all detergents in the trade for correct implementation of classification, labelling and packaging of finished products¹⁹.

However, the methodology to assess the safety properties of finished products through a calculation method was not applied here. Had the manufacturer or Inveresk undertaken this method and amalgamated the safety properties of the individual ingredients in the paint (i.e. the existing LC₅₀ data), then it would have become apparent that the concentration of the paint used in this acute toxicity experiment was above and beyond that required to kill the rats, on the basis of two main ingredients alone.

Alternatives – use of advanced techniques

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. Anti-fouling ships paints are currently causing environmental concern as they dissolve slowly into water and can cause pollution^{12,13,14}.

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.
- Using human tissue samples² 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 shown to predict inhaled formaldehyde toxicity and could be developed for use with other substances.

CONCLUSIONS

The major aims of this report were to:

- emphasise the importance of a vote against the inclusion of an additional animal acute toxicity test in Annex V;
- to highlight the necessity to stringently apply the criteria set out in Annex IX to avoid the unnecessary use of animals; and
- to emphasise the importance of **enforcement** of legislation.

It also successfully highlights the importance of avoiding animal tests wherever possible, and of using advanced, non-animal techniques to obtain more relevant human data, such as those put forward by MEP Caroline Lucas.

A major issue which has become apparent from the revelations from Inveresk is that the chemicals industry produces documents of poor quality, full of errors and inconsistencies. It is now clear that, in at least some cases, the actual animal experiments which they conduct or procure are **poorly designed** and **sloppily reported**.

Before Government 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. Before they vote on the inclusion of an animal test for acute toxicity in Annex V, they also need to question the detrimental after-effects, for both humans and animals, of doing so, in light of the evidence presented here.

OUR BACKGROUND

Animal Defenders International, 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).

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