Department of Health and Social Security

Report on Health and Social Subjects

30

GUIDANCE ON THE PREPARATION OF SUMMARIES OF DATA ON CHEMICALS IN FOOD, CONSUMER PRODUCTS AND THE ENVIRONMENT SUBMITTED TO DHSS

Her Majesty's Stationery Office

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Contents

	Page
Preface	iv
1. **Introduction**	1
2. **Guidance on the compilation of the summary document**	3
Appendix 1: Sample submission	7

Preface

Toxicological data on a variety of chemicals used in industry, consumer products or food, or appearing as contaminants in food or the environment, are submitted to DHSS, either directly or through other Government Departments, for evaluation. Until now, the secretariats to the expert committees which advise Government on these matters have prepared summaries of the original data and these, together with any comments the secretariats may have, are presented to the committees for their consideration.

After my predecessor, Sir Henry Yellowlees, had held a meeting with industry to discuss the shortage of toxicologists, particularly in Government, a Working Group was established to consider how to facilitate the processing of data submitted. It was agreed that considerable time might be saved if industry were to provide summaries of data. This would also give industry an opportunity to present their views on the interpretation of the data. It will not, of course, obviate the need for the complete data to be submitted, or for the secretariats to check the summaries and comment as necessary.

This document, which incorporates the views of industry, other Government Departments and the expert advisory committees, offers guidance on the preparation of summaries of data. Whilst it is not intended to be a checklist, nor to replace the detailed reports of studies, I do hope that it will go some way toward speeding up the consideration of submissions.

E D ACHESON

Chief Medical Officer
Department of Health
and Social Security

1986

1. Introduction

When a chemical is referred for advice on its safety-in-use to one of the DHSS's independent committees of experts on toxicity, carcinogenicity and mutagenicity, the Departmental secretariat prepares a detailed summary of the original data together with comments for consideration by members of the committee. To expedite the progress of submissions it has been suggested that it would be of great assistance both to the Department and to industry if the submitter could produce the detailed summary until now prepared by the various secretariats. This would also give an opportunity for submitters to offer their own evaluation of the data.

This document is intended to provide general guidance concerning the presentation of summaries. It is not intended to be a check-list, and neither would the inclusion of such a summary in a submission alter the need to submit full detailed reports of studies.

For guidance on the principles of investigating toxicity, carcinogenicity or mutagenicity, the reader is referred to the Guidelines prepared by the relevant Committees (Guidelines for the testing of chemicals for toxicity[1]; Guidelines for the testing of chemicals for carcinogenicity[2]; Guidelines for the testing of chemicals for mutagenicity[3]). The main part of this document gives general information applicable to the majority of toxicity reports submitted; an example of a submission including summaries of four individual types of study is given in Appendix 1.

The summary is essentially a detailed précis of the submitted data which should be comprehensive and which may include both published and unpublished studies. It is intended to highlight the pertinent findings including the absence of significant effects. It is particularly important that control and negative data are included. Attention should be drawn to any particular weaknesses or limitations in the data. Data on the actual or expected exposure to, or intake of the chemical should be provided. Summaries of individual studies should cross-refer to the relevant volume and page numbers in the original data submitted. Inclusion of a statement on whether the data submitted were generated in accordance with the accepted principles of Good Laboratory Practice would also be helpful. It is recommended that these summaries should be compiled by people experienced in the interpretation of toxicological data. A summary document should preferably not exceed 30 pages in length.

References

1 Department of Health and Social Security. *Guidelines for the testing of chemicals for toxicity*. London: HMSO, 1982. (Report on health and social subjects; 27).

2 Department of Health and Social Security. *Guidelines for the testing of chemicals for carcinogenicity*. London: HMSO, 1982. (Report on health and social subjects; 25).
3 Department of Health and Social Security. *Guidelines for the testing of chemicals for mutagenicity*. London: HMSO, 1981. (Report on health and social subjects; 24).

2. Guidance on the Compilation of the Summary Document

1. Title

This should do nothing more than identify the substance or product and its intended use; eg

i. ethylene oxide—for use as a sterilant for surgical dressings
ii. hydrogenated glucose syrup—for use as a sweetener in food instead of sucrose

2. Technical data

i. Name
   —names in the IUPAC nomenclature
   —other names (usual name, trade names, synonyms)
   —grade (eg technical or food grade)
   —CAS number (if available)
ii. Specification of the substance or product being submitted including:
   —composition by %, w/v or ppm
   —empirical and structural formula } of each component
   —molecular weight }
   —purity
   —known impurities and percentage of main impurities
   —physical form (liquid, powder etc)
   —solubility—eg aqueous, organic solvents, lipid
   —other information—eg vapour pressure at appropriate temperature
   specific gravity (if doses specified in units of volume)
   important salts
   pKa (where appropriate)
   hydrolysis data
   particle size, distribution of powders etc
   stability under given storage conditions
   —outline of manufacturing process (in detail if a material cannot be specified in any other way).
iii. Method(s) of detection and their sensitivity.
iv. Intended use, fields of application and possible combinations with other materials.
v. Likely human exposure (actual or expected), including:
   —route, frequency, duration and other factors influencing exposure

— maximum and average dosage or exposure
— variations affecting particular sections of the population (eg by age, sex, disease or occupation)
— exposure from other sources
— residues in foods

vi. Environmental distribution, where appropriate, dealing in broad terms with likely distribution of the chemical (eg pesticide) and final disposal (eg effluent).

3. Studies submitted, their objectives and rationales

This section should list the studies submitted and outline briefly their objectives and rationales. The reasons for carrying out unusual studies (eg elucidation of the mechanism of an effect observed in other studies) should be stated. Equally the reasons should be stated for not submitting a study of a type that might be expected.

4. Sections 1–3 above need only be completed once for each substance or product. However sections 4a, b and c below need to be completed for each study submitted.

Each experiment should be summarized in the order listed in Section 3.

The following information should be provided:

a. *Experimental design*

i. material tested—plus specification if different from that given in Section 2 (eg technical grade rather than food grade), batch number if appropriate.

ii. animals—species, strain, microbiological status, sex, random allocation to groups, group sizes, age and/or weight at the start of the study, type of diet, antimicrobial agents, other drugs or vaccines administered.

iii. compound preparation and administration—route, duration, frequency, dosage (by volume as well as concentration for liquids), estimated total doses (for long-term studies), controls (positive and negative) and vehicle.

iv. duration of study if different from duration of dosage.

v. any unusual study design considerations such as paired feeding, satellite groups or interim kills should be noted.

vi. nature of major observations—eg frequency of body weight measurement, timing, volume and site of blood sampling, timing of urine collection, haematological and clinical chemistry parameters measured, method of killing animals, organs weighed, organs examined macroscopically/microscopically, methods of examining fetuses, etc.

vii. statistical analyses employed—specify methods used including reference to publication of any new or unusual statistical tests used.

b. *Results*

This section should be clear and concise. The main findings should be summarized and a statement made on whether significant deviations from control and normal values occurred. It is helpful if data, including the information outlined below, are presented in the following order:

i. clinical condition—general health, intercurrent disease, behaviour (including the results of specific observations if undertaken), ophthalmoscopic findings
ii. mortality—times of death, and causes where known
iii. weight change, food and water consumption, relationship of growth rates to dosage and to any changes in food consumption/utilization
iv. haematology
v. clinical chemistry
vi. pathology—the macroscopic abnormalities observed at post-mortem examination and abnormalities observed on histological examination should be described. In addition to the current control incidence of lesions, the background incidence may need to be given, eg the incidence of tumours in control groups in other experiments carried out in the same strain and sex at a similar time (concurrent controls) or in the past (historical controls). This is of particular importance when it appears that the current control data are atypical. Where special studies (histochemistry, electron microscopy or quantitative histology) have been undertaken the findings should be described in relation to those from routine histology.

c. *Comment*

The significant findings from the study should be highlighted together with the no-adverse-effect level, if one has been determined, and any other relevant information.

d. *Reference*

Author, journal and date (published data) or submitter, laboratory and date (unpublished data).

5. Special studies

Special studies carried out for a specific purpose should be summarized as above but other information may be required.

eg. *Sensitization studies*

As techniques vary considerably, the summary should state the reasons for choosing the particular method used (this may simply be because of previous experience with it) and detail the methods used for induction and challenge (eg number and timing of doses, sites of administration, vehicle or adjuvant used), the concentrations employed, reasons for their choice, the interval between induction and challenge and method of grading the results.

6. Review of results and conclusions

The document should list all significant findings in all studies and seek to make interpretations and draw conclusions, which the submitter would wish the Committee/Department to consider. Reasons for disregarding any findings should be carefully stated. Where relevant, attention may be drawn to the extent to which dosage or concentrations are exaggerated and the possible influence of the vehicle used for administering the substance. The conclusion should include an interpretation of the significance of the findings in terms of possible mechanisms of the effect seen in the animal and extrapolation of the animal data to humans. References to known effects (or lack of effect) in human exposure should be given; evidence from recorded experience for occupational exposure, for example, may be informative. The evaluation of potential human hazard should be made in the context of known or likely human exposure, including that from other sources.

7. Author of the summary

Appendix 1 — Sample Submission

1. Title

B gas — a monomer in plastics and rubber manufacture.

2. Technical data

i. B gas, synonym C gas, Tradename X;
ii. Specification:

composition	— of known mixtures (where applicable)
chemical formula	— C_xH_y and structure
molecular weight	— Z
purity	— 97%
known impurities	— D-1.5%, E-1%
physical form	— supplied as a liquid under pressure
solubility	— a% solubility in water, b% in acetone
method of generating atmosphere	— vaporized in a heat exchanger at 30°C. After trapping to remove BC (inhibitor) and dimers, the gas was mixed with filtered air to the required concentration. Concentrations analysed and monitored continuously by chromatography.

iii. Intended uses: a monomer in the plastics and rubber industry, particularly in combination with monomer S to produce a copolymer T.
iv. Likely human exposure: occupational exposure of males and females at an 8 hour weighted average level of 5 ppm (with peak levels of 50 ppm). Minimal exposure of general public via residual monomer in plastic and rubber materials in consumer products etc.
v. Environmental distribution: not appropriate.

3. Types of studies submitted, their objectives and rationales, and whether they were conducted in accordance with the principles of Good Laboratory Practice.

i. A long-term carcinogenicity study in the rat was performed because of potential long-term human exposure from occupational sources or from residues in consumer products.
ii. A teratogenicity study in the rat was performed because females may be occupationally exposed to this gas.

iii. A mutagenicity study in *Salmonella typhimurium* and a cytogenetic study in rat bone marrow cells were carried out because of possible risks to those exposed occupationally.
iv. A metabolism study in the rat was carried out to ascertain whether there was any potential for accumulation of the gas with repeated exposure.

4.1 Long-term Carcinogenicity Study in the Rat

a. *Experimental design*

i. Material tested: B gas (specification as given in Section 2)
ii. Animals:
- —Sprague Dawley rats, CD strain, obtained from . . ., with groups of 10 animals of each sex screened for specific micro-organisms before the study started.
- —110 rats/sex/group, rats allocated randomly.
- —average age three weeks, weight 50–70 g at the start of the study.
- —pelleted diet, manufactured by . . ., and water available *ad libitum*, when rats not in exposure chambers.

iii. Compound administration:
- —By inhalation for six hours per day for five days a week for two years, at levels of 0, 1000 and 8000 ppm, 10 animals per group were killed at 52 weeks, the remainder at 111 weeks (males) or 105 weeks (females). The rats were exposed in stainless steel and glass chambers, measuring $2 \times 2 \times 2$ m; filtered air (95% at a particle size of 1μm) was fed into the chambers at 1000 L/min (minimum 800 L/min) entering at the top via a baffle, to aid mixing. B gas was supplied in pressurized cylinders, vaporized in water jackets at 30°C and fed through traps to remove the polymer inhibitor (BC) and dimers. The chamber concentration was monitored by an IR gas analyser, each chamber having nine sampling points. The controls were exposed to filtered air only.

iv. Unusual study design features: None.
v. Major observations:
- —Animals were weighed weekly for the first 3 months and then monthly thereafter.
- —Blood samples collected from the orbital sinus from groups of 20 animals per sex per group (pre-selected) at weeks 13, 26, 52 and 78 and examined for red blood cell count (RBC), haemoglobin (Hb), packed cell volume (PCV), reticulocytes, white blood cell count (WBC), plus differential and platelet count. Supplemented by further groups of 10 at 52 weeks because of leucocytosis at 26 weeks.
- —Blood samples for clinical chemistry were collected from separate animals as described for haematology and analysed for blood urea nitrogen (BUN), glucose, serum alanine aminotransferase (ALT), serum aspartate aminotransferase (AST), serum alkaline phosphatase (AP), total protein, albumin/globulin ratio and electrolytes (Na, K, Cl).
- —Rats were killed by exsanguination under barbiturate anaesthesia and examined macroscopically. Absolute and relative (to bodyweight)

weights of the following organs were recorded: brain, heart, liver, spleen, kidneys, adrenals, gonads and lungs. Samples of the above organs plus those listed below were prepared for histological examination: aorta, caecum, colon, duodenum, eyes, ileum, lymph nodes, mammary glands, muscle, nasal cavity, oesophagus, pancreas, pituitary, prostate, rectum, salivary glands, sciatic nerve, seminal vesicles, skin, stomach, thymus, thyroid, tongue, trachea, urinary bladder and uterus. A sample of bone marrow was also removed from the femur.

vi. Statistics:
—Survival data by Peto's analysis (Peto and Pike, 1973. *Biometrics* **29** 579).
—Bodyweight, haematology and clinical chemistry data by analysis of variance.
—Tumour data were analysed as recommended by Peto *et al.* (Peto, Pike, Day, Gray, Lee, Parish, Peto, Richards and Wahrendorf, 1980. *IARC Monographs*. Supp 2. 327).

b. *Results*

i. Clinical condition:
—Red nasal and lachrymal secretion common over the first five weeks of the study in the treated groups and towards the end of the experiment in all animals. Loss of hair towards the end of the study in all animals.

ii. Mortality data
—Mortality in males at the end of the study was 55% in the control group, 50% in the low dose group and 68% in the top dose group. The corresponding figures in females were 54%, 68% and 76%. There was a significant difference in mortality rate between treated males and females at both doses and between the low and high dose groups of both sexes. Mortality rates of high dose males and low and high dose females were significantly increased in comparison with controls.

iii. Weight gain
—No treatment-related differences overall, but all test animals showed a reduced weight gain during the first two weeks which was later regained.

iv. Food and water consumption:
—Not monitored.

v. Haematology
—There was a significant increase in WBC in female test groups compared to the control from week 26; however, this was attributed to unusually low WBC in the control group. This was substantiated by the provision of background data from other studies carried out recently in the laboratory. There were a few random but statistically significant differences between test and control groups in PCV and RBC but these were not considered to be related to treatment because there was no clear dose-response relationship. All values remained within the historical normal range.

vi. Clinical chemistry
Blood
—There were a few statistically significant differences between test and control groups but all values remained within the normal range and were not considered to be related to treatment.
Urine
—Not monitored.

vii. Pathology
—There were no significant differences in organ weights that could be related to treatment. There was an increase in mammary gland tumours in the treated females (13–23%) compared to the control group (10%) but no dose-relationship was apparent. One urinary bladder transitional cell papilloma and one astrocytoma were seen in the top dose group males and one uterine adenocarcinoma in the top dose group females; none of these tumour types was seen in the control group. For all other tumours seen, the incidence was comparable in the test and control groups.

c. *Comment*

There were no explanations for the dose- and sex-related differences in mortality. B gas produced a slight, but not dose-related, increase in the incidence of mammary gland tumours in treated groups compared to the controls. Also various tumours were seen, albeit at a low incidence in the high dose group rats, which were not observed in the control group. However all of these tumours are known to occur spontaneously in this strain of rat, with an incidence comparable to that seen in the high dose group in this study (as shown by background data for this strain of rat in this laboratory), and their occurrence was not thought to be related to treatment.

B gas would therefore not appear to be carcinogenic to the rat following exposure by inhalation to 8000 ppm for six hours/day for two years.

d. *Reference*

Smith and Jones (1982).

4.2 Teratogenicity Study in the Rat

a. *Experimental design*

i. Material tested: B gas (specification as given in Section 2).

ii. Animals:
—Sprague Dawley rats, CD strain, obtained from . . .
—40 females in the control group, 24 females in each of the test and positive control groups. Virgin females of weight range 210–270 g were received, quarantined for 14 days and then mated overnight (one male to two females). The females were checked by vaginal smears and those in late oestrus and with sperm detected were designated day 0 of pregnancy and randomly allocated to the various groups.

—pelleted diet, manufactured by . . ., and water available *ad libitum*, when the rats were not in exposure chambers.

iii. Compound administration:
—By inhalation for six hours per day; on days 6 to 15 inclusive of gestation at levels of 0, 200, 1000 and 8000 ppm. The rats were exposed in stainless steel and glass chambers as described in the long-term study above. The negative controls were exposed to filtered air in chambers as described above. The positive controls received 250 mg/kg/day acetylsalicylic acid by gavage in distilled water on days 6 to 15 inclusive of gestation.

iv. Unusual study design features:
—None.

v. Major observations:
—Bodyweight of dams measured daily. Dams killed on day 20 by cervical dislocation. Numbers of fetuses (live and dead), numbers of early and late resorptions, numbers of corpora lutea, fetal weights, crown-rump lengths, fetal sex noted. All fetuses examined for external abnormalities, then half stained with Alizarin Red S for skeletal examination and half examined for soft tissue abnormalities by Wilson slicing.

vi. Statistics:
—Bodyweight data by analysis of variance, other data by non-parametric tests (refer to which tests).

b. *Results*

i. Clinical condition:
—All animals (including controls) showed red nasal and lachrymal secretions and alopecia. The positive controls showed some respiratory distress and increased occurrence of alopecia.

ii. Mortality data:
—No deaths.

iii. Bodyweight gain:
—There was a significant reduction in weight gain in the top dose group (10%) and in the positive control group (15%).

iv. Food and water consumption:
—Not monitored.

v. Teratological observations:
—The pregnancy incidence was between 90–100% in all groups.

The numbers of corpora lutea and implantations were similar in all groups but there was a significant increase in resorption rate in the positive control group only (15% compared to 2% in control group).

Fetal weights were slightly reduced in the top dose group as were the crown-rump lengths. Gravid uterine weight was also decreased in the top dose group only. There was an increased incidence of delayed ossification in the top dose group only. The expected malformations were seen in the positive control group (cleft palate, missing limbs and delayed ossification).

c. *Comment*

Although the incidence of major malformations and minor anomalies was not affected by exposure to B gas, a slight decrease in fetal weight and size was noted in the top dose group only (8000 ppm), together with a delay in ossification in these fetuses. Maternal weight gain was reduced at this exposure level and the fetal effects may therefore have been secondary to the toxicity seen in the mothers and not due to a direct toxic effect on the fetus. Exposure to 1000 ppm B gas for six hours/day during the period of organogenesis was without effect on the fetus or mother.

d. *Reference*

Smith and Jones (1981).

4.3 Mutagenicity tests

4.3.1 In Bacteria

a. *Experimental design*

i. Material tested:
—B gas (specification as given in Section 2).

ii. Test system:
—*Salmonella typhimurium* strains TA 1535 and TA 100 (for base pair substitutions) and TA 1537, TA 1538 and TA 98 (for frameshift mutations), both with and without metabolic activation using rat liver S9 fraction obtained from rats pretreated with Aroclor.

iii. Compound administration:
—Dose range-finding studies were carried out in gassing jars equipped with stirrers at 0, 500, 5000, 25000 and 50000 ppm for six hours in the presence and absence of S9. B gas was toxic to the tester strains at 50000 ppm in the absence and presence of S9. The experiments were therefore carried out by direct plate assays in the gassing jars in atmospheres containing 0, 1000, 2000, 4000, 8000, 16000 and 32000 ppm B gas for six hours at 37°C after which the plates were placed in incubators for 72 hours.
—Each experiment was carried out using triplicate plates. B gas was supplied in pressurized cylinders, vaporized in water jackets at 30°C and fed through traps to remove the polymer inhibitor (BC) and dimers. The concentration in the gassing jar was monitored by an IR gas analyser. The positive and negative controls were exposed to filtered air only. Sodium azide at 5 μg/plate was used as a positive control for strains TA 1535 and TA 100, 9-aminoacridine at 20 μg/plate for strain TA 1537 and 2-nitrofluorene at 10 μg/plate for strains TA 1538 and TA 98 without metabolic activation. 2-anthramine at 2 μg/plate was used as the positive control for all strains in the plates grown with the metabolic activation system. All strains were checked for histidine requirement and ampicillin resistance, as appropriate, prior to use, and had spontaneous revertant rates within the expected frequency range.

iv. Statistics:
—Student's t-test.

b. *Results*

In the first experiment B gas was toxic to the bacteria in all plates at 32000 ppm but there was no reduction in the bacterial lawn at 16000 ppm and below, in the absence of S9 activation. In the presence of S9, there was some reduction in the bacterial lawn at doses down to and including 8000 ppm. B gas produced a small increase in the number of revertants in the presence of S9 at 8000 and 16000 ppm (approximately 2-fold increase at each dose). In a repeat experiment B gas exposure levels of 4000, 6000, 8000, 12000 and 16000 ppm were chosen. There was no indication of cytotoxicity at 4000, 6000 and 8000 ppm but there was a slight reduction in the background lawn at 12000 ppm and a marked reduction in bacterial background lawn at 16000 ppm in the presence of S9. There was no sign of toxicity in the absence of S9. Once again there was a small increase in the number of revertants in the presence of S9 at 16000 ppm (2-fold increase) and just over a 50% increase in the number of revertants at 8000 and 12000 ppm. There was no increase in the number of revertants at any dose in the absence of S9.

c. *Comments*

There was a small increase in the number of revertants, which was not dose-related, in the presence of S9 at doses of B gas above 6000 ppm. This correlated well with evidence of toxicity of B gas, as demonstrated by a reduction in bacterial lawn, and this effect therefore was probably due to non-specific cytotoxicity rather than a demonstration of mutagenicity of B gas. In conclusion B gas was not mutagenic at exposures up to 16000 ppm in the absence of S9. Minimal mutagenicity was demonstrated only when cytotoxicity was evident at exposures over 6000 ppm in the presence of S9. Positive controls produced the expected elevated mutation frequencies.

d. *Reference*

Bloggs et al. (1980).

4.3.2 Cytogenetic Studies in Mammalian Cells In Vivo

a. *Experimental design*

i. Material tested:
—B gas (specification as given in Section 2).

ii. Animals:
—Sprague Dawley rats, CD strain, obtained from . . .
—Five animals/sex/group/sampling period, animals allocated randomly.

iii. Compound administration:
—Preliminary toxicological data showed that 16000 ppm was the maximum dose level tolerated by the rats for six hours without producing marked irritation to the nose and eyes. Rats were exposed

once only to B gas by inhalation for six hours at levels of 0, 1000, 8000 and 16000 ppm. Positive and negative controls were exposed to filtered air only. The positive controls received a single dose of mitomycin C (4 mg/kg) at the end of the exposure period.

Two hours before death the rats were injected intraperitoneally with colchicine (4 mg/kg body weight) to obtain an adequate number of cells in metaphase. The rats were killed 6, 24 and 48 hours after the end of the exposure period. Marrow was collected from the femurs and 50 cells in metaphase per animal examined for aberrations. These were recorded as percentage cells with aberrations and aberrations per cell. Gaps were recorded separately. The mitotic index was established for each animal.

iv. Statistics:
— A doubling above the background rate of chromosome aberrations in controls was taken to be significant.

b. *Results*

There was no significant increase in the incidence of chromosomal aberrations detected. At the top dose level, which caused a slight reduction in mitotic index, there was a 3% incidence of cells with aberrations compared to 3.5% incidence in the negative control group (0.5% and 0.53% respectively when gaps were excluded).

c. *Comments*

B gas did not show any evidence of mutagenic activity when administered by inhalation at exposures up to 16000 ppm for six hours.

d. *Reference*

Bloggs et al (1981).

4.4 Metabolism Study in the Rat

a. *Experimental design*

i. Material tested:
— B gas (specification as given in Section 2).

ii. Animals:
— Sprague Dawley rats, CD strain, obtained from . . .
— Nine rats/sex/group, three killed at the end of the six hour exposure period and three at 24 and 48 hours post-exposure. Similarly, further groups of three rats/sex/dose group were killed 0, 24 and 48 hours after exposure to B gas by inhalation for seven days for six hours/day. Samples of blood, liver, kidney and fat were analysed for B.
— Pelleted diet, manufactured by . . ., and water available *ad libitum* when rats not in exposure chambers.

iii. Compound administration:
— By inhalation for six hours per day for one or seven days, at levels

of 0, 1000 and 8000 ppm. The rats were exposed in stainless steel and glass cages as described in the long-term study above. The controls were exposed to filtered air only.

iv. Statistics:
—Student's t-test.

b. *Results*

i. Clinical condition:
—Red nasal and lachrymal secretions seen in treated animals.

ii. Metabolic data:
—Blood levels were similar after one or seven days exposure to B gas with a rapid decline within 24 hours post-exposure. At 48 hours virtually no chemical B was detected in the blood (limit of detection 2 μg/ml). Peak levels seen in females were somewhat higher than in males.

Group	Sex	Exposure level	Mean level of chemical B (μg/ml) hours post-exposure 0	24	48
Single dose	M	1000	40	10	2
	F		55	15	3
	M	8000	150	40	5
	F		200	55	6
7 days exposure	M	1000	45	12	3
	F		60	17	3
	M	8000	160	43	6
	F		215	60	7

—Chemical B was detected in the liver and kidneys in small amounts immediately after a single exposure (8 and 5 μg/g tissue) in the top dose group but larger amounts were seen in the fat (25 μg/g in the high dose groups and 10 μg/g in the low dose group). Levels in the liver and kidney were negligible after 24 hours (approx 1 μg/g—this being the limit of detection) but levels declined more slowly from the fat with levels of 5 and 3 μg/g still present 48 hours post-exposure. Repeated exposure for seven days resulted in slightly higher levels in the fat (35 and 15 μg/g in the high and low dose groups respectively) but levels in the liver and kidney were similar to those seen after single exposure.

c. *Comment*

Levels of chemical B in the blood, liver and kidneys were similar after single or repeated exposure to B gas, and declined rapidly. However there was some evidence of accumulation of chemical B in fat after repeated exposure.

d. *Reference*

Smith and Jones (1980).

5. Special studies

None.

6. Review and conclusions

The studies performed do not provide any evidence that B gas is carcinogenic when administered to rats by inhalation daily for two years, at levels up to 8000 ppm. Bacterial mutagenicity tests were negative, as were *in vivo* cytogenetic studies using rat bone marrow cells. Teratogenicity studies showed delayed fetal development accompanied by reduced maternal weight gain at 8000 ppm only but no teratogenic effects were seen. Metabolism studies provided some evidence for accumulation of chemical B in the fat on repeated exposure.

There is unlikely to be any hazard to workers exposed occupationally to B gas. Current occupational exposure levels are between 20 and 100 ppm, the present long-term exposure limit (formerly Threshold Limit Value) being 1000 ppm.

7. Author

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