FOOD STANDARDS
Australia New Zealand
Te Mana Kounga Kai – Ahitereiria me Aotearoa

12 December 2007
[8-07]

DRAFT ASSESSMENT REPORT

APPLICATION A602

EXTRANEOUS RESIDUE LIMIT (PARADICHLOROBENZENE)

DEADLINE FOR PUBLIC SUBMISSIONS: 6pm (Canberra time) 6 February 2008
SUBMISSIONS RECEIVED AFTER THIS DEADLINE WILL NOT BE CONSIDERED
(See 'Invitation for Public Submissions' for details)

For Information on matters relating to this Assessment Report or the assessment process generally, please refer to http://www.foodstandards.gov.au/standardsdevelopment/

Executive Summary

Standard 1.4.2 – Maximum Residue Limits of the *Australia New Zealand Food Standards Code* (the Code) stipulates the requirements for residues of agricultural and veterinary chemicals in food. The Standard includes a separate Schedule for Extraneous Residue Limits (ERLs). An ERL is the maximum permitted limit of a pesticide residue, arising from environmental sources other than the use of a pesticide directly or indirectly on the food.

An Application was received from the Australian Honey Bee Industry Council (AHBIC) on 21 March 2007 to include an ERL for paradichlorobenzene in honey in Schedule 2 of Standard 1.4.2. The Applicant requested a limit of 0.1 mg/kg for a finite period of five years. This limit is the same as the default limit[1] that the New Zealand Food Safety Authority has used to manage residues of paradichlorobenzene in honey in New Zealand.

The *Agreement between the Government of Australia and the Government of New Zealand concerning a Joint Food Standards System* (the Treaty), excludes the establishment of maximum residue limits for agricultural and veterinary chemicals in food from the joint food standards setting system. Australia and New Zealand independently and separately develop maximum residue limits for agricultural and veterinary chemicals in food.

In assessing residues of agricultural and veterinary chemical residues in food, FSANZ's primary concern is the safety of the residues. FSANZ will not approve limits for residues in the Code if the residues associated with the limits represent an unacceptable risk to public health and safety. For the reasons stated below, FSANZ considers that incorporating an ERL in the Code for paradichlorobenzene in honey would be an appropriate interim measure to allow implicated honey to be sold while industry eliminates the residues of paradichlorobenzene from honey.

Purpose

The purpose of the Application is to vary Schedule 2 of Standard 1.4.2 – Maximum Residue Limits to include an ERL of 0.1 mg/kg for paradichlorobenzene in honey for a period of five years.

Preferred Approach

It is proposed to vary Schedule 2 of Standard 1.4.2 – Maximum Residue Limits to include a temporary ERL of 0.1 mg/kg for paradichlorobenzene in honey. It is further proposed that the limit cease to have effect five years from gazettal, although FSANZ will review the limit sooner if advised that it is no longer required or if States and Territories advise FSANZ that the limit is promoting misuse of paradichlorobenzene containing products.

[1] All imported and domestically-produced food sold in New Zealand (except for food imported from Australia) must comply with the New Zealand (Maximum Residue Limits of Agricultural Compounds) Food Standards (the MRL Standards) and amendments. The MRL Standards list the MRLs for a range of agricultural compounds, but also include a provision for residues of up to 0.1 mg/kg for agricultural compound/food combinations not specifically listed.

Reasons for Preferred Approach

- no public health or safety concerns have been identified in relation to the residues associated with the proposed ERL (the dietary exposure was assessed as being less than 0.1% of the tolerable daily intake);

- until recently (April 2007), paradichlorobenzene was approved for use in stored bee hives to control wax moth. Based on the data and information available, wax in previously treated hives may contain residues of paradichlorobenzene and this could result in low levels of residues in honey from these hives;

- while only some honey has been found to contain low levels of residues, the inclusion of the limit would allow apiarists to sell this honey while they identify and replace implicated hives in an orderly and economically viable timeframe;

- incorporating either a Maximum Level (contaminant) or a Maximum Residue Limit (veterinary chemical) would be inappropriate; given the veterinary chemical history for paradichlorobenzene and that its use as a veterinary chemical is no longer permitted;

- the limit is consistent with the residue data provided by the Applicant and is consistent with the limit and overall approach used by New Zealand authorities to manage such residues in honey in New Zealand;

- a temporary limit is proposed to apply for five years to reflect the interim nature of the ERL while the industry ultimately eliminates residues of paradichlorobenzene from honey. This timeframe seems reasonable given the current drought and consequently, the number of hives in storage;

- the limit will be reviewed earlier than this if it was no longer considered necessary or if FSANZ were advised by States and Territories that the limit was promoting misuse of paradichlorobenzene containing products; and

- not incorporating an ERL for paradichlorobenzene in honey would mean that any implicated honey could not be legally sold even though the residues are of low public health significance.

Consultation

Under section 36 of the *Food Standards Australia New Zealand Act 1991* (FSANZ Act), FSANZ decided not to invite public submissions in relation to the Application prior to making a Draft Assessment. It was satisfied that the Application raised issues of minor significance or complexity only.

Although FSANZ decided not to invite public submissions prior to making a Draft Assessment, a number of organisations provided comment on the Initial Assessment Report for this Application. In the interests of transparency, the issues raised in this correspondence have been considered by FSANZ in preparing this Draft Assessment Report. FSANZ now invites written submissions for the purpose of the Final Assessment and will have regard to any submissions received.

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INVITATION FOR PUBLIC SUBMISSIONS

FSANZ invites public comment on this Draft Assessment Report based on regulation impact principles and the draft variation to the Code for the purpose of preparing an amendment to the Code for approval by the FSANZ Board.

Written submissions are invited from interested individuals and organisations to assist FSANZ in preparing the Final Assessment of this Application. Submissions should, where possible, address the objectives of FSANZ as set out in section 18 of the FSANZ Act. Information providing details of potential costs and benefits of the proposed change to the Code from stakeholders is highly desirable. Claims made in submissions should be supported wherever possible by referencing or including relevant studies, research findings, trials, surveys etc. Technical information should be in sufficient detail to allow independent scientific assessment.

The processes of FSANZ are open to public scrutiny, and any submissions received will ordinarily be placed on the public register of FSANZ and made available for inspection. If you wish any information contained in a submission to remain confidential to FSANZ, you should clearly identify the sensitive information and provide justification for treating it as confidential commercial information. Section 114 of the FSANZ Act requires FSANZ to treat in-confidence, trade secrets relating to food and any other information relating to food, the commercial value of which would be, or could reasonably be expected to be, destroyed or diminished by disclosure.

Submissions must be made in writing and should clearly be marked with the word 'Submission' and quote the correct project number and name. Submissions may be sent to one of the following addresses:

Food Standards Australia New Zealand
PO Box 7186
Canberra BC ACT 2610
AUSTRALIA
Tel (02) 6271 2222
www.foodstandards.gov.au

Food Standards Australia New Zealand
PO Box 10559
The Terrace WELLINGTON 6036
NEW ZEALAND
Tel (04) 473 9942
www.foodstandards.govt.nz

Submissions need to be received by FSANZ by 6pm (Canberra time) 6 February 2008.

Submissions received after this date will not be considered, unless agreement for an extension has been given prior to this closing date. Agreement to an extension of time will only be given if extraordinary circumstances warrant an extension to the submission period. Any agreed extension will be notified on the FSANZ website and will apply to all submitters.

While FSANZ accepts submissions in hard copy to our offices, it is more convenient and quicker to receive submissions electronically through the FSANZ website using the Standards Development tab and then through Documents for Public Comment. Questions relating to making submissions or the application process can be directed to the Standards Management Officer at the above address or by emailing standards.management@foodstandards.gov.au.

Assessment reports are available for viewing and downloading from the FSANZ website. Alternatively, requests for paper copies of reports or other general inquiries can be directed to FSANZ's Information Officer at either of the above addresses or by emailing info@foodstandards.gov.au.

INTRODUCTION

An Application was received from the Australian Honey Bee Industry Council (AHBIC) on 21 March 2007 to include an Extraneous Residue Limit (ERL) of 0.1 mg/kg for paradichlorobenzene in honey in Schedule 2 of Standard 1.4.2 – Maximum Residue Limits of the Code for five years.

The term 'paradichlorobenzene' has been used throughout this Report because it is the term commonly used in the industry and this term has been used to assist in promoting consultation on this Application. The alternative expression for paradichlorobenzene is '1,4- dichlorobenzene' and this is the nomenclature used in the draft variation.

1. Background

1.1 Current Standard

Standard 1.4.2 stipulates the requirements for residues of agricultural and veterinary chemicals in food, including for Extraneous Residue Limits (ERLs). An ERL is the maximum permitted limit of a pesticide residue, arising from environmental sources other than the use of a pesticide directly or indirectly on the food. Schedule 2 lists all ERLs in particular foods.

If an ERL for an agricultural chemical in a food is not listed in Schedule 2 there must be no detectable residues of that agricultural chemical in that food. Currently, there are no specific limits for paradichlorobenzene in any food in Standard 1.4.2 and therefore no detectable residues of paradichlorobenzene are permitted in any food.

The *Agreement between the Government of Australia and the Government of New Zealand concerning a Joint Food Standards System* (the Treaty), excludes the establishment of maximum residue limits for agricultural and veterinary chemicals in food from the joint food standards setting system. Australia and New Zealand independently and separately develop MRLs for agricultural and veterinary chemicals in food. The ERL proposed by the Applicant is the same as the default limit[2] that the New Zealand Food Safety Authority (NZFSA) has used to manage residues of paradichlorobenzene in honey in New Zealand.

1.2 Historical Background

Paradichlorobenzene is a chlorinated organic substance. According to the Applicant, the presence of paradichlorobenzene in honey stems from the historical use of this chemical for the fumigant insecticide control of wax moth in stored combs.

FSANZ has been advised that approval for the use of paradichlorobenzene was recently withdrawn (April, 2007) and that prior to this, the use was only formally approved in Queensland.

[2] All imported and domestically-produced food sold in New Zealand (except for food imported from Australia) must comply with the New Zealand (Maximum Residue Limits of Agricultural Compounds) Food Standards (the MRL Standards) and amendments. The MRL Standards list the MRLs for a range of agricultural compounds, but also include a provision for residues of up to 0.1 mg/kg for agricultural compound/food combinations not specifically listed.

The instructions for use of paradichlorobenzene (PDB) in Queensland stated:

> *For protection of equipment during periods of storage against possibility of infestation.*
>
> 1. *Fumigate all spare bee boxes and combs with phosphine.*
> 2. *All combs are to be free of honey.*
> 3. *Stack bee boxes up to 5 high and secure seal all joints. Spread 100 g of P.D.B. crystals on a paper or cloth sheet (30 cm × 30 cm) laid on top bars of the frames in the top box.*
> 4. *Seal top.*
> 5. *Repeat application after crystals have vapourised (3-4 weeks in summer, longer in winter).*
> 6. *Ventilate treated boxes for minimum of 24 hours before use.*

FSANZ has been advised that no MRL was established for paradichlorobenzene in honey and it has been suggested to FSANZ that legitimate use with ventilation as described above did not produce detectable residues in the honey. FSANZ has considered this previously approved use in assessing the available data and information on residues of paradichlorobenzene in honey.

1.3 Trans Tasman Mutual Recognition Arrangement

The Trans Tasman Mutual Recognition Arrangement (TTMRA) between Australia and New Zealand commenced on 1 May 1998. The following provisions apply under the TTMRA.

- Food produced or imported into Australia that complies with Standard 1.4.2 of the Code can be legally sold in New Zealand.

- Food produced or imported into New Zealand that complies with the New Zealand (Maximum Residue Limits of Agricultural Compounds) Food Standards, 2007 (and amendments) can be legally sold in Australia.

2. The Issue

The industry was alerted to the problem of paradichlorobenzene residues in honey when residues above 0.01 mg/kg were detected by European authorities[3] in honey from Australia. While paradichlorobenzene use is no longer approved, it has been suggested that low level residues have been found in honey from some producers as a result of the leaching of residues from waxes or other components in previously treated hives.

The industry is concerned about primary producers who may be unaware of the past use of paradichlorobenzene and may still be using older previously treated hives for honey production.

[3] Under Regulation (EC) No 396/2005 of the European Parliament and of the Council of 23 February 2005 on maximum residue levels of pesticides in products of plant and animal origin, the maximum pesticide residue level in foodstuffs is 0.01 mg/kg. This general limit is applicable 'by default', i.e. in all cases where an MRL has not been specifically set for a product or product type.

Honey containing paradichlorobenzene from these producers will not be accepted by honey processors unless a limit is established to recognise the presence of these residues in honey.

The Applicant's proposed limit of 0.1 mg/kg is consistent with the limit in food legislation in New Zealand and the Applicant proposed that the limit apply for a finite period of five years, to implement the industry strategy for phasing out residues. See **Attachment 5** for a copy of this strategy.

3. Objectives

The objective of this assessment is to ensure that any amendment of Standard 1.4.2 is consistent with the protection of public health and safety, and the legitimate use of agricultural and veterinary chemical products.

In developing or varying a food standard, FSANZ is required by its legislation to meet three primary objectives which are set out in section 18 of the FSANZ Act. These are:

- the protection of public health and safety;
- the provision of adequate information relating to food to enable consumers to make informed choices; and
- the prevention of misleading or deceptive conduct.

In developing and varying standards, FSANZ must also have regard to:

- the need for standards to be based on risk analysis using the best available scientific evidence;
- the promotion of consistency between domestic and international food standards;
- the desirability of an efficient and internationally competitive food industry;
- the promotion of fair trading in food; and
- any written policy guidelines formulated by the Ministerial Council.

The Ministerial Council has endorsed a Policy Guideline for the Regulation of Residues of Agricultural and Veterinary Chemicals in Food, which has now been provided to FSANZ. In consultation with stakeholders, FSANZ will explore alternative options for regulating chemical residues in food. To ensure appropriate consultation, this process will take some time to complete.

The proposed draft variation to Standard 1.4.2 is consistent with FSANZ's section 18 objectives of food regulatory measures, including the Ministerial Policy Guidelines on the Regulation of Residues of Agricultural and Veterinary Chemicals in Food[4].

[4] http://www.health.gov.au/internet/wcms/publishing.nsf/Content/2087CDEAEE7C703CCA256F190003AF4B/$File/pol-g-line-reg-res.pdf

4. Key Assessment Questions

The key questions considered as part of this assessment are:

- Do the residues of paradichlorobenzene at the proposed limit pose any public health and safety issues?
- Is the limit proposed by the Applicant appropriate and achievable?
- Is the proposed timeframe for the limit of five years acceptable and appropriate?

RISK ASSESSMENT

5.1 Hazard Assessment Summary

When exposed to air, paradichlorobenzene (alternatively known as 1,4-dichlorobenzene) slowly sublimates from a solid to a vapour. It is the vapour that acts as a deodorizer or insect repellent. Most people recognize the odour of paradichlorobenzene as the smell of mothballs or urinal deodorant, and can smell it in the air at relatively low levels. To facilitate a dietary risk assessment for paradichlorobenzene when present in honey the toxicological hazard was characterised in order to establish a tolerable daily intake (TDI) (**Attachment 2**).

Paradichlorobenzene is of low acute toxicity by the oral, dermal and inhalation routes but is a slight skin and eye irritant. It also irritates the respiratory tract and is a weak dermal sensitiser. In humans and laboratory animals, the principal target organs are the liver, kidneys and respiratory, nervous and haematopoietic systems. Liver injury occurs in most laboratory species, and is accompanied in mice by development of benign and malignant hepatocellular tumours arising from sustained increases in the rates of cellular metabolism, death and proliferation. Kidney injury occurs in mice, rats and dogs, with male rats being susceptible to renal tubular cell adenocarcinoma arising from a specific lesion caused by interaction between paradichlorobenzene and α_{2u}-globulin. The weight of evidence indicates that paradichlorobenzene is not genotoxic. Due to well characterised differences in modes of detoxification between rodents and humans, there is a negligible likelihood that liver or kidney cancers will occur in humans exposed to low doses of paradichlorobenzene in the diet.

Studies in rats and rabbits have shown that while paradichlorobenzene is toxic to the developing foetus and/or neonates, this occurs only at doses high enough to cause toxicity to the mother. The chemical does not cause developmental malformations or impair male or female fertility and mating.

The lowest NOEL in studies with paradichlorobenzene in experimental animals is 10 mg/kg bw/day, observed in a 12-month dog study by oral administration. The most suitable safety factor for human risk assessment is 100, comprised of 10-fold factors for inter-species extrapolation and within-species [human] variation. Therefore, the TDI for paradichlorobenzene is 0.1 mg/kg bw/day. The margins between the TDI and NOELs for adrenal, liver and kidney tumours in rodents are 3000, 1200 and 540, respectively.

5.2 Dietary Exposure Assessment

A dietary exposure assessment for paradichlorobenzene was undertaken for the Australian and New Zealand populations with a focus on Australian children aged 2-6 because children generally have the highest dietary exposure relative to all other age groups because of their higher food consumption per kilogram of body weight (**Attachment 3**).

The food consumption data used were from the 1995 Australian National Nutrition Survey (NNS) and the 1997 New Zealand NNS. The concentration data for paradichlorobenzene in honey was from the Application and was at the proposed ERL of 0.1 mg/kg.

For consumers of honey, the estimated mean paradichlorobenzene dietary exposure for the Australian population aged 2 years and above was 1.3 µg/day. The estimated 90th percentile dietary exposure to paradichlorobenzene was 2.9 µg/day. Seventeen percent (17%) of the Australian population aged 2 years and above were consumers of honey and products, and honey in mixed dishes.

The estimated mean exposures to paradichlorobenzene in honey were higher for the New Zealand population aged 15 years and above (1.7 µg/day) than for Australians aged 2 years and above (1.3 µg/day). The estimated 90th percentile dietary exposure to paradichlorobenzene for the New Zealand population was approximately 3.9 µg/day. As for Australia, approximately 17% of the New Zealand population were reported to be consumers of honey and products, and honey in mixed dishes.

For Australian children aged 2-6 years, the estimated dietary exposure to paradichlorobenzene for honey consumers (0.7 µg/day) was less than that for Australians aged 2 years and above (1.3 µg/day) and New Zealanders aged 15 years and above (1.7 µg/day). The 90th percentile exposures were also lower for Australian children aged 2-6 years (1.6 µg/day).

In order to determine whether the level of estimated dietary exposures to paradichlorobenzene will be of concern to public health and safety, exposures were compared to a TDI of 0.1 mg/kg bw/day which was established by FSANZ. The estimated dietary exposures to paradichlorobenzene were well below (<0.1%) the TDI for all population groups.

5.3 Risk Characterisation

The conclusions from the Safety Assessment (section 5.1) and Dietary Exposure Assessment (section 5.2 and **Attachment 3**) analysis are as follows:

- although toxicological concerns have been identified following high oral exposure of paradichlorobenzene in laboratory rodents, this appears to be a species-specific effect, with no evidence of a public health and safety concern for humans;
- FSANZ established a TDI of 0.1 mg/kg bw/day which is considered to be a safe level of exposure for humans;
- for the highest exposed population subgroup, namely 2-6 year old children, the exposure was less than 0.1% of the TDI; and
- at this level of exposure there are unlikely to be any health and safety concern for all population subgroups.

RISK MANAGEMENT

Standard 1.4.2 stipulates the requirements for residues of agricultural and veterinary chemicals in food, including for Extraneous Residue Limits (ERLs). An ERL is the maximum permitted limit of a pesticide residue, arising from environmental sources other than the use of a pesticide directly or indirectly on the food. Schedule 2 lists all ERLs in particular foods.

If an ERL for an agricultural chemical in a food is not listed in Schedule 2 there must be no detectable residues of that agricultural chemical in that food. Currently, there are no specific limits for paradichlorobenzene in any food in Standard 1.4.2 and therefore no detectable residues of paradichlorobenzene are permitted in any food.

6. Limit proposed by the Applicant

FSANZ has taken into account a number of issues in assessing this Application and considers that the key issues for this Application are:

1. the safety of the residues of paradichlorobenzene reported in honey;
2. whether it is appropriate to legitimise the presence of these residues in honey for an interim period while honey producers identify implicated producers, institute corrective action and ultimately eliminate residues from honey;
3. the mechanism for instituting this interim measure;
4. the practicality of any proposed limit; and
5. the relevant timeframe for which the regulatory measure should apply.

The safety of the residues associated with the ERL has been reported above in Section 5.

6.1 Residues in Honey

FSANZ has considered the studies and other documents available on residues of paradichlorobenzene in honey (**Attachment 6**). These studies and documents are primarily based upon laboratory experiments and theoretical calculations and assumptions, rather than any specific practical experimental data on honey, wax, combs or honeycomb.

The Applicant also provided some monitoring data indicating that waxes in implicated hives contain residues of paradichlorobenzene. The three results for residues of paradichlorobenzene in beeswax were 2.62, 3.12 and 3.22 mg/kg. Given the lipophilic nature of paradichlorobenzene, the higher residues in beeswax compared to honey are not unexpected. These data confirm that the wax in hives is a potential source of the residues in honey.

Until recently (April 2007), paradichlorobenzene was approved for use to control wax moths in bee hives. The available information suggests that residues of paradichlorobenzene are likely to occur in honey produced in hives that have been treated with paradichlorobenzene. This is based on the capacity of the wax to absorb paradichlorobenzene, its retention in the wax despite any aeration, and the subsequent leaching of paradichlorobenzene from the wax into honey that comes into contact with the wax.

However, the data and information are not sufficient to determine the level of residues that would result from the previously approved use.

FSANZ considers that residue limits should be included in the Code where the residues result from the legitimise use of chemical products. Based upon the data and information available, it is possible that residues could have resulted from the legitimate historical use of paradichlorobenzene. On this basis, FSANZ considers that it would be appropriate to consider establishing a limit for paradichlorobenzene in honey to recognise the residues resulting from this use.

FSANZ invites comment on whether it is appropriate in this situation to establish a limit to allow the sale of honey containing low level residues of paradichlorobenzene.

6.2 Appropriate Food Regulatory Measure

FSANZ has considered three food regulatory measure options, namely an ERL as proposed by the Applicant, a Maximum Residue Limit (MRL) or a contaminant Maximum Level (ML). In considering the appropriate food regulatory measure, FSANZ has noted that:

- paradichlorobenzene containing products should not be sold for use in apiary situations (e.g. hives);
- paradichlorobenzene containing products should not be used to control pests or diseases under State and Territory 'control of use' legislation[5];
- paradichlorobenzene has previously been approved as a veterinary chemical for treatment of hives in Queensland;
- the existence of other ERLs (e.g. Dieldrin, DDT) has not promoted the misuse of products containing these chemicals;
- beeswax is part of the food production system for honey and in some cases beeswax can be used for many years in a hive, with hives being transferred from person to person and from generation to generation of apiarists. On this basis, the beeswax in a hive has similarities with food production structures and fixtures in other agricultural situations that have also been found to contain long since deregistered chemicals (e.g. dieldrin), and where residues may occur in food derived from these facilities;
- according to the Applicant, the residues have resulted from past use resulting in paradichlorobenzene in the wax in hives; and
- a general interpretation of the term 'environmental sources' would include the structures within the hives, including the wax.

As defined in the Code, an ERL is appropriate where residues are 'arising from environmental sources other than the use of a pesticide directly or indirectly on the food.' As 'environmental sources' are not defined, it is possible to interpret this expression in a general sense, which in turn would mean that the term could be used to encapsulate the fixtures and components of a hive treated in accordance with former veterinary use. On this basis, FSANZ considers that an ERL would be an appropriate food regulatory measure to address residues resulting from previously treated hive fixtures.

[5] 'Control of use' legislation is the State and Territory legislation that regulates the use of agricultural and veterinary chemical products after they are sold.

FSANZ acknowledges that this is a broad interpretation of the term 'environmental sources' but also considers that this interpretation is not inconsistent with the definition of an ERL.

Incorporating an ERL would be a more restrictive measure than an MRL, as the source of the residues is limited to 'environmental sources' and not from the direct or indirect use of a chemical product on a food. Incorporating an ERL would also be consistent with the approach of reserving an MRL for residues arising from the contemporary approved use of an agricultural or veterinary chemical. Given the past use of paradichlorobenzene as a veterinary chemical, it would be inappropriate to consider residues of it as a contaminant and to use an ML as the food regulatory measure. FSANZ therefore considers that an ERL, rather than an MRL or ML, would be an appropriate food regulatory measure and that this is consistent with the approach taken with residues of other 'deregistered' organochlorines (e.g. DDT).

FSANZ is aware of some concerns about the incorporation of an ERL for paradichlorobenzene in honey. If an ERL was not incorporated then this would mean that honey containing the residues of paradichlorobenzene could not be legally sold and implicated producers would need to dispose of their honey. FSANZ is of the view that this would be a strict approach to take with these producers and their associated communities when:

- no public health and safety concerns have been identified with the residues associated with the ERL;

- based on the best available scientific evidence, the residues could conceivably (but not definitively) have resulted from the legitimate historical use of an approved chemical product; and

- the industry is only seeking the food regulatory measure to allow the sale of the implicated honey for an interim period while it institutes corrective action to eliminate the residues. The industry has developed a strategy for eliminating paradichlorobenzene residues from honey (**Attachment 5**).

In summary, if it is considered appropriate for honey containing low level paradichlorobenzene residues to be sold then the view of FSANZ is that the only practical and most appropriate measure for achieving this is by incorporating a temporary ERL for paradichlorobenzene in honey in the Code. Incorporating either an ML or an MRL would be inappropriate; given the veterinary chemical history for paradichlorobenzene and that its use as a veterinary chemical in apiary situations is no longer permitted. Recognising that the ERL is an interim measure and consistent with the approach used for other interim residue limits in Standard 1.4.2, a temporary ERL is considered appropriate.

FSANZ invites comment on whether an MRL or some other food regulatory measure is more appropriate for managing paradichlorobenzene residues in honey.

FSANZ specifically invites comment on the implications for 'control of use' agencies[6] if a temporary ERL for paradichlorobenzene in honey were to be incorporated in the Code.

[6] 'Control of use' agencies are those agencies responsible for regulating the use of agricultural and veterinary chemical products after they are sold.

6.3 Practical Compliance with the Proposed Limit

The Applicant has proposed a limit of 0.1 mg/kg and provided monitoring data stating that residues between 0.091 mg/kg and 0.01 mg/kg (limit of reporting) have been reported in honey (**Attachment 6**). Based on the data provided by the Applicant, only a proportion of honey samples have been found to contain residues and a limit of 0.1 mg/kg would be sufficient to account for the highest residues reported in honey.

The Applicant also provided monitoring data indicating the decline in residues in implicated apiaries over a period of time. Based on the data (**Attachment 6**), the rate of decline appears to vary from apiary to apiary but in general terms the concentration appears to halve over a two month period for those apiaries with residues above 0.03 mg/kg. For those apiaries with residues initially under 0.03 mg/kg, the residues generally reduce to below detectable residues in two months. While these data indicate a rate of decline to undetectable residues in two months, it must also be recognised that the limit would need to apply for sufficient time to allow implicated hives to be identified. For example, implicated hives currently in storage because of drought may only be identified once these hives are brought into production. Following this, sufficient time will be needed to allow the honey from these hives to be sold while corrective action is taken to eliminate the residues.

In considering the proposed limit, FSANZ has taken into account the industry practice of blending of honey. Blending implicated honey with non-implicated honey would reduce the residues in the final bended honey product. Despite this, the limit for paradichlorobenzene in honey must be high enough to account for residues in implicated honey that has not been blended. This is necessary to ensure that implicated honey can be 'handled for sale'[7] during production and processing and prior to blending.

As previously stated, the data provided by the Applicant indicated that a limit of 0.1 mg/kg would be sufficient to account for the highest residues reported in honey. Furthermore, the Bogdanov study[8] (**Attachment 6**) indicated that residues of paradichlorobenzene detected in honey are for the most part under 0.1 mg/kg. FSANZ has also noted that a limit of 0.1 mg/kg has been used by New Zealand authorities to manage paradichlorobenzene residues in honey. On the basis of all this information, FSANZ considers that a limit of 0.1 mg/kg for paradichlorobenzene in honey is the most appropriate limit.

It has been suggested to FSANZ that it may be necessary to review whether a limit of 0.01 mg/kg or 0.1 mg/kg is appropriate. In considering this point, the primary concerns for FSANZ are the safety of the residues associated with the limit and the ability to comply with any limit. An assessment of the safety of the residues has been reported above in Section 5. Based on the data provided by the Applicant, the implicated honey would not comply with a limit of 0.01 mg/kg. On this basis, FSANZ sees little value in incorporating a limit in the Code which will not address the problem that the industry is facing. FSANZ has also noted that incorporating the proposed limit of 0.1 mg/kg would be consistent with the approach used in New Zealand and would therefore result in a consistent approach to managing residues of paradichlorobenzene in honey from a Trans-Tasman perspective.

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[7] Food legislation regulates food for sale and food handled for sale i.e. the preparation of food prior to sale.

[8] Bogdanov S, Kilchenmann V, Seiler K, Pferrerli H, Frey Th, Roux B, Wenk P, Noser J. Residues of para-dichlorobenzene in honey and beeswax. Journal of Apicultural Research, 43(1): 14-16 (2004)

Overall, the data and information provided by the Applicant indicate that a limit of 0.1 mg/kg would be achievable for implicated honey producers and would ensure consistency with the approach taken in New Zealand. It should be noted that incorporating a limit of 0.1 mg/kg would only be relevant in Australia and would have limited or no relevance in other countries.

FSANZ invites comment on whether a limit of 0.1 mg/kg is achievable, including any additional data indicating the levels of paradichlorobenzene residues in honey.

The Applicant has indicated that methods exist for measuring paradichlorobenzene in honey and that compliance with the limit can be practically monitored.

FSANZ invites comment on the availability of methods for determining compliance with the proposed limit.

6.4 Proposed Timeframe for the Limit

The Applicant has requested that the proposed limit apply for a finite period of five years. This has been proposed by the Applicant to fully implement the industry strategy for phasing out residues of paradichlorobenzene in honey (**Attachment 5**).

Time-limited provisions have previously been used by FSANZ to manage residues of substances in food (e.g. ethylene oxide). According to the Food and Agriculture Organization document *Submission and evaluation of pesticide residues data for the estimation of maximum residue levels in food and feed, Rome, 2002,* extraneous maximum residue limits should be reviewed every five years. On the basis of the international practice, FSANZ proposes that the ERL apply for a period of five years.

The ERL can be reviewed at any time in the five year period and FSANZ would review the limit if States and Territories advised FSANZ that the limit was promoting misuse of paradichlorobenzene containing products.

6.5 Monitoring of Residues

In other correspondence, issues were raised in relation to investigating misuse, monitoring residues and monitoring the progress of the industry's strategy for eliminating residues. FSANZ understands that the honey industry is funding testing under the National Residue Survey and these results will be published when available. FSANZ understands that there are national forums where compliance and monitoring issues can be discussed and where plans may be developed for mitigating potential misuse and monitoring the elimination of the residues. If considered necessary and consistent with its statutory role, FSANZ would be prepared to assist in the consideration of these issues.

7. Options

FSANZ is required to consider the impact of various regulatory (and non-regulatory) options on all sections of the community, including consumers, food industries and governments. The regulatory options available for this Application are:

7.1 Option 1 – To not vary the Code to incorporate an ERL for paradichlorobenzene in honey

This option maintains the *status quo* by requiring the residues of paradichlorobenzene to be undetectable in honey.

7.2 Option 2 – Vary the Code to incorporate an ERL of 0.1 mg/kg for paradichlorobenzene in honey for a period of five years

This option would require an amendment to Schedule 2 of Standard 1.4.2 to incorporate an ERL that would allow honey containing residues of paradichlorobenzene up to 0.1 mg/kg to be legally sold for five years.

8. Impact Analysis

8.1 Affected Parties

The parties likely to be affected by this Application include:

1. honey producers and processors may benefit from the variation as it would enable them to supply and process honey containing low levels of paradichlorobenzene;

2. consumers that may have concerns about residues in honey and those consumers who may benefit in relation to supply of honey, although this benefit is unlikely to be discernable; and

3. government agencies in Australia who ensure compliance with the Code, on the basis that these agencies may need to develop methods for monitoring paradichlorobenzene in honey.

8.2 Benefit Cost Analysis

8.2.1 Option 1 – To not vary the Code to incorporate an ERL for paradichlorobenzene in honey

8.2.1.1 Benefits

- there are no perceived or discernable benefits for affected parties if this option is adopted.

8.2.1.2 Costs

- for consumers, there are unlikely to be any discernable costs as the unavailability of some honey from certain producers and processors is likely to be seen as typical seasonal fluctuation in the supply.

FSANZ invites comment on whether these costs are likely to be discernable by consumers.

- for some producers and processors of honey, adopting this option would result in costs resulting from not being able to legally sell honey containing residues of paradichlorobenzene. These costs could include cessation of beekeeping by some producers as the costs of immediate hive decontamination may not be viable for these producers. The cessation of these activities may also have an impact on pollination services which complement honey production activities in certain regions.

- some honey processors may also be affected with reduced supply to the packing sector and the inability to meet market or contract commitments. Given the nature of beekeeping and the historical use of paradichlorobenzene, the costs of this option are most likely to be borne by smaller producers and communities in rural and regional areas of Australia that in many cases are already bearing the production costs of drought on their business.

- for Australian Government, State and Territory agencies, adopting this option would be unlikely to result in any specific costs.

8.2.2 Option 2 – Vary the Code to incorporate an ERL of 0.1 mg/kg for paradichlorobenzene in honey for a period of five years

8.2.2.1 Benefits

- for consumers, the major benefit would be maintaining existing confidence in the food supply in relation to residues of agricultural and veterinary chemicals by recognising the residues occurring in honey through an open and transparent process.

- for some producers and processors of honey, adopting this option would allow these businesses to remain viable through the sale of honey containing low level residues of paradichlorobenzene, while they identify implicated hives and eliminate residues over a finite period of time.

- for Australian Government, State and Territory agencies, adopting this option would foster community confidence that regulatory authorities are maintaining standards to minimise residues in the food supply, while being reasonable and responsive about inadvertent residues that may be detected in food.

8.2.2.2 Costs

- for consumers, there are unlikely to be any discernable costs if a limit were included in the Code.

FSANZ invites comment on whether there are any costs that are likely to be discernable by consumers.

- for producers and processors of honey, adopting this option is unlikely to result in major costs.

FSANZ invites comment on whether there are any costs for honey producers and processors associated with incorporating a limit for paradichlorobenzene in honey.

- for Australian Government, State and Territory agencies, adopting this option would be unlikely to result in any specific costs, although there may be a need to develop specific analytical methods for paradichlorobenzene in honey to monitor compliance with the proposed limit.

8.3 Comparison of Options

In assessing Applications, FSANZ considers the impact of various regulatory (and non-regulatory) options on all sectors of the community, including consumers, food industries and governments in Australia.

For Application A602, Option 1 is a viable but undesirable option because of the potentially substantial costs to certain honey producers and processors which may impact negatively on their viability and in turn the viability of the rural and regional communities that depend upon the sale of their produce or their pollination services. These costs are considered to be substantially more than the costs to the community of short-term exposure to residues that are of minimal public health significance.

Option 2 is a viable and desirable option as its assists implicated honey producers in addressing the residues in honey in an economically viable timeframe.

The conclusion of the impact analysis is that the benefits of Option 2 (incorporating the proposed temporary ERL in the Code for a period of five years) would outweigh the associated costs.

COMMUNICATION AND CONSULTATION

9. Communication

This Application requests that an ERL of 0.1 mg/kg for paradichlorobenzene in honey be included in Schedule 2 of Standard 1.4.2 for five years.

FSANZ has applied a basic communication strategy to Application A602. This will involve advertising the availability of the Draft Assessment Report for public comment in the national press and making the reports available on the FSANZ website. During the assessment process, FSANZ will consult key stakeholders.

The Applicant, individuals and organisations that make submissions on this Application will be notified at each stage of the Application. If the FSANZ Board approves the draft variation to the Code, FSANZ will notify the Ministerial Council of its decision. The Applicant and stakeholders, including the public, will be notified on the gazettal of changes to the Code in the national press and on the FSANZ website.

10. Consultation

10.1 Public Consultation

FSANZ decided, pursuant to section 36 of the FSANZ Act not to invite public submissions in relation to the Application prior to making a Draft Assessment. However, FSANZ invites written submissions for the purpose of the Final Assessment under paragraph 17(3)(c) of the FSANZ Act and will have regard to submissions received. FSANZ made its decision under section 36 because it was satisfied that the Application raised issues of minor significance or complexity only.

Section 63 of the FSANZ Act provides that, subject to the *Administrative Appeals Tribunal Act 1975*, an application for review of FSANZ's decision to omit to invite public submissions prior to making a Draft Assessment, may be made to the Administrative Appeals Tribunal.

Although FSANZ decided not to invite public submissions prior to making a Draft Assessment, a number of organisations provided comment on the Initial Assessment Report. In the interests of transparency, the issues raised in this correspondence have been considered in developing this Draft Assessment Report and the FSANZ response to these issues have been tabulated in **Attachment 4**.

FSANZ now invites written submissions for the purpose of the Final Assessment and will have regard to any submissions received.

10.2 World Trade Organization (WTO)

As members of the World Trade Organization (WTO), Australia is obligated to notify WTO member nations where proposed mandatory regulatory measures are inconsistent with any existing or imminent international standards and the proposed measure may have a significant effect on trade.

There are no relevant international standards relevant to this Application and amending the Code to include an ERL for paradichlorobenzene in honey is unlikely to have a significant effect on international trade as the inclusion of the limit provides more flexibility for honey producers than if the limit were not included.

Given the past trade disruptions that have occurred in trade of honey containing paradichlorobenzene residues, notification will be recommended to the agencies responsible in accordance with Australia's obligations under the WTO Technical Barriers to Trade (TBT) Agreement. This will enable other WTO member countries to comment on proposed changes to standards where they may have a significant impact on them.

CONCLUSION

11. Conclusion and Preferred Approach

Preferred Approach

It is proposed to vary Schedule 2 of Standard 1.4.2 – Maximum Residue Limits to include a temporary ERL of 0.1 mg/kg for paradichlorobenzene in honey. It is further proposed that the limit cease to have effect five years from gazettal, although FSANZ will review the limit sooner if advised that the limit is no longer required or if States and Territories advise FSANZ that the limit is promoting misuse of paradichlorobenzene containing products.

11.1 Reasons for Preferred Approach

- no public health or safety concerns have been identified in relation to the residues associated with the proposed ERL (the dietary exposure was assessed as being less than 0.1% of the tolerable daily intake);

- until recently, paradichlorobenzene was approved for use in stored bee hives to control wax moth. Based on the data and information available, wax in previously treated hives may contain residues of paradichlorobenzene and this could result in low levels of residues in honey from these hives;

- while only some honey has been found to contain low levels of residues, the inclusion of the limit would allow apiarists to sell this honey while they identify and replace implicated hives in an orderly and economically viable timeframe;

- incorporating either a Maximum Level (contaminant) or a Maximum Residue Limit (veterinary chemical) would be inappropriate; given the veterinary chemical history for paradichlorobenzene and that its use as a veterinary chemical is no longer permitted;

- the limit is consistent with the residue data provided by the Applicant and is consistent with the limit and overall approach used by New Zealand authorities to manage these residues in honey in New Zealand;

- a temporary limit is proposed to apply for five years to reflect the interim nature of the ERL while the industry ultimately eliminates residues of paradichlorobenzene from honey. This timeframe seems reasonable given the current drought and consequently, the number of hives in storage;

- the limit will be reviewed earlier than this if it was no longer considered necessary or if FSANZ were advised by States and Territories that the limit was promoting misuse of paradichlorobenzene containing products; and

- not incorporating an ERL for paradichlorobenzene in honey would mean that any implicated honey could not be legally sold even though the residues are of low public health significance.

12. Implementation and Review

Given that residues of paradichlorobenzene in honey have been detected, it is proposed that the Extraneous Residue Limit (ERL) come into effect upon gazettal.

Reflecting the interim need for the ERL, it is further proposed that the limit cease to have effect five years from gazettal.

ATTACHMENTS

1. Draft variation to the *Australia New Zealand Food Standards Code*
2. Safety Assessment Report
3. Dietary Exposure Assessment Report
4. Summary of Other Correspondence
5. Industry Paradichlorobenzene Strategy
6. Paradichlorobenzene in Honey Information

Attachment 1

Draft variation to the *Australia New Zealand Food Standards Code*

Standards or variations to standards are considered to be legislative instruments for the purposes of the Legislative Instruments Act (2003) and are not subject to disallowance or sunsetting.

To commence: on gazettal

[1] ***Standard 1.4.2*** *of the Australia New Zealand Food Standards Code is varied by inserting in Schedule 2 –*

1,4-DICHLOROBENZENE 1,4-DICHLOROBENZENE	
HONEY	TE0.1

To commence: 5 years from gazettal

[2] ***Standard 1.4.2*** *of the Australia New Zealand Food Standards Code is varied by omitting from Schedule 2 –*

1,4-DICHLOROBENZENE 1,4-DICHLOROBENZENE	
HONEY	TE0.1

Attachment 2

Safety Assessment Report

Executive Summary

In laboratory animals paradichlorobenzene (alternatively known as 1,4-dichlorobenzene), a volatile and fat-soluble chemical, is rapidly and extensively absorbed by the gastrointestinal tract and lungs. The highest tissue levels are attained in adipose tissue. Humans and laboratory species metabolise paradichlorobenzene in the liver to form an epoxide that binds to proteins or DNA, or is further metabolised. The parent chemical and its metabolites are excreted rapidly, mainly via the kidneys.

Paradichlorobenzene is of low acute toxicity by the oral, dermal and inhalation routes but is a slight skin and eye irritant. It also irritates the respiratory tract and is a weak dermal sensitiser. In humans and laboratory animals, the principal target organs are the liver, kidneys and respiratory, nervous and haematopoietic systems. Acute and repeated exposure at relatively high doses can cause tremors, weakness and unconsciousness in laboratory species, and hyporeflexia, ataxia and impaired speech and movement in humans. Haemolytic anaemia and/or methaemoglobinaemia have been reported in industrial workers and domestic users of paradichlorobenzene products, while microcytic anaemia, reduced WBC counts and hyper- or hypoplasia of the bone marrow or spleen have been observed in laboratory species. When exposed chronically by inhalation, rats and mice display injury and/or metaplasia of cells lining the respiratory or olfactory passages, possibly arising from irritation.

Liver injury occurs in most laboratory species, and is accompanied in mice by development of benign and malignant hepatocellular tumours arising from sustained increases in the rates of cellular metabolism, death and proliferation. Kidney injury occurs in mice, rats and dogs, with male rats being susceptible to renal tubular cell adenocarcinoma arising from a specific lesion caused by interaction between paradichlorobenzene and α_{2u}-globulin. The weight of evidence indicates that paradichlorobenzene is not genotoxic. Due to well characterised differences in modes of detoxification between rodents and humans, there is a negligible likelihood that liver or kidney cancers will occur in humans exposed to low doses of paradichlorobenzene in the diet. Dietary exposure of humans is also unlikely to cause the development of adrenal gland tumours, which occur in male mice exposed chronically at high doses.

Studies in rats and rabbits have shown that while paradichlorobenzene is toxic to the developing foetus and/or neonates, this occurs only at doses high enough to cause toxicity to the mother. The chemical does not cause developmental malformations or impair male or female fertility and mating.

The lowest NOEL in studies with paradichlorobenzene in experimental animals is 10 mg/kg bw/d, observed in a 12-month dog study by oral administration. The most suitable safety factor for human risk assessment is 100, comprised of 10-fold factors for inter-species extrapolation and within-species [human] variation. Therefore, the TDI for paradichlorobenzene is 0.1 mg/kg bw/d. The margins between the TDI and NOELs for adrenal, liver and kidney tumours in mice and rats are 3000, 1200 and 540, respectively.

INDEX

GLOSSARY OF ABBREVIATIONS

Time

d	Day
h	Hour
min	Minute
mo	Month
wk	Week
s	Second
yr	Year

Weight

bw	Body weight
g	Gram
kg	Kilogram
µg	Microgram
mg	Milligram
ng	Nanogram
wt	Weight

Length

cm	Centimetre
m	Metre
µm	Micrometre
mm	Millimetre
nm	Nanometre

Dosing

id	Intradermal
im	Intramuscular
inh	Inhalation
ip	Intraperitoneal
iv	Intravenous
po	Oral
sc	Subcutaneous
mg/kg bw/d	mg/kg bodyweight/day

Volume

L	Litre
mL	Millilitre
µL	Microlitre

Concentration

M	Molar
ppb	Parts per billion
ppm	Parts per million

Clinical chemistry, haematology

AAP	Alanine aminopeptidase
A/G	Albumin/globulin ratio
ALT	Alanine aminotransferase (SGPT)
AP	Alkaline phosphatase
AST	Aspartate aminotransferase (SGOT)
BUN	Blood urea nitrogen
CPK	Creatine phosphatase (phosphokinase)
FSH	Follicle stimulating hormone
GGT	Gamma-glutamyl transferase
GSH	Glutathione
Hb	Haemoglobin
Hct	Haematocrit
IU	International units
LDH	Lactate dehydrogenase
LH	Luteinising hormone
MCH	Mean corpuscular haemoglobin
MCHC	Mean corpuscular haemoglobin concentration
MCV	Mean corpuscular volume
NAG	B-N-acetylglucosaminidase
NTE	Neurotoxic target esterase
PCV	Packed cell volume (Haematocrit)
PT	Prothrombin time
RBC	Red blood cell/erythrocyte
T_3	Triiodothyroxine
T_4	Thyroxine
TSH	Thyroid stimulating hormone (thyrotropin)
WBC	White blood cell/leucocyte
WBC-DC	White blood cells – differential count

Anatomy

CNS	Central nervous system
GIT	Gastro-intestinal tract

Chemistry

DMSO	Dimethyl sulfoxide
ECD	Electron capture detection
GC	Gas chromatography
GLC	Gas liquid chromatography
HPLC	High pressure liquid chromatography
LOD	Limit of detection
LOQ	Limit of quantification
MS	Mass spectrometry
NMR	Nuclear magnetic resonance
RIA	Radioimmunoassay
TLC	Thin layer chromatography
UV	Ultraviolet

Terminology

ADI	Acceptable Daily Intake
ARfD	Acute Reference Dose
GD	Gestation Day
GLP	Good Laboratory Practice
LD	Lactation Day
LOEC	Lowest Observed Effect Concentration
LOEL	Lowest Observed Effect Level
MOE	Margin of Exposure
MRL	Maximum Residue Limit or Level
NOEC	No Observed Effect Concentration
NOEL	No Observed Effect Level
NOAEL	No Observed Adverse Effect Level
PND	Post Natal day
TDI	Tolerable Daily Intake

Organisations & publications

CAC	Codex Alimentarius Commission
ECETOC	European Chemical Industry Ecology and Toxicology Centre
FAO	Food and Agriculture Organisation of the UN
FSANZ	Food Standards Australia New Zealand
IARC	International Agency for Research on Cancer
IPCS	International Programme on Chemical Safety
JECFA	FAO/WHO Joint Expert Committee on Food Additives
JMPR	Joint Meeting on Pesticide Residues
NCI	National Cancer Institute
NTP	National Toxicology Program
US EPA	United States Environmental Protection Agency
WHO	World Health Organisation

INTRODUCTION

Low level residues of paradichlorobenzene (alternatively known as 1,4-dichlorobenzene) have been found in honey from some producers as a result of the 'leaching' of residues from contaminated waxes in hives. According to the Applicant, this contamination has occurred as a result of the previously approved and historical use of paradichlorobenzene.

A risk assessment of pesticides which may remain in food as trace residues usually requires the establishment of a health standard such as an acceptable daily dose (ADI). An ADI is derived from a toxicological database in which a variety of laboratory animals have been dosed with the test compounds at many multiples of the likely human exposure. The purpose of such studies, which include testing many important toxicological endpoints, such as cancer induction and effects on reproduction, is to identify a dose at which no adverse effects are observed. This dose, which is then further reduced to account for intra-and interspecies variability, forms the basis of the ADI. The same health standard setting process can also be applied to naturally occurring environmental or other non-environmental contaminants such as paradichlorobenzene. The only difference will be the name of the health standard; Tolerable Daily Intake (TDI).

This review of the mammalian toxicology and metabolism/toxicokinetics of paradichlorobenzene was undertaken in order to establish a TDI. The core sources of data reviewed here were the WHO (1991) Environmental Health Criteria Report No. 128 (*Chlorobenzenes other than hexachlorobenzene*), US NTP (1987) Technical Report on the toxicology and carcinogenesis of paradichlorobenzene, and relevant papers published in the scientific literature. These have been supplemented where necessary with evaluations of additional studies from the EU (2004) Risk Assessment Report on paradichlorobenzene and the US EPA (2006) draft *Toxicological Review of Dichlorobenzenes*.

EVALUATION AND RECOMMENDATION

Database Adequacy

The toxicological database on paradichlorobenzene is extensive but of variable quality, due partly to the age of some of its constituent studies. Nevertheless, the data set has proven adequate to allow a TDI to be derived, based on subchronic exposure studies in rats and mice, a chronic study in dogs, chronic/carcinogenicity studies in rats and mice, two-generation reproduction studies in rats, and developmental toxicity studies in rats and rabbits.

Due to paradichlorobenzene being a volatile commodity chemical to which industrial workers and consumers are exposed mainly by inhalation, some major long-term and developmental studies in laboratory animals have been performed via both the oral and inhalation routes. However, given that the chemical is not intended for use in ways that would lead to dietary exposure of humans, study designs and dose selection have not been optimised towards providing data suitable for use in the risk assessment being performed in this report. Several pivotal studies (especially those originating from the US NTP) did not demonstrate NOELs for significant toxicological end-points, including cancer. These issues, nevertheless, are ameliorated by the large body of genotoxicity and mechanistic data relevant to paradichlorobenzene's modes of carcinogenic activity in rats and mice. This has facilitated judgements as to the relevance to humans of the findings in laboratory rodents, and informed the choice of the safety factor that has been used to derive a TDI.

Metabolism and toxicokinetics

Paradichlorobenzene is quite extensively and rapidly absorbed by the gastrointestinal tract and lungs, with oral and pulmonary uptakes of *ca* 75% and 50 – 70% in laboratory species. Peak levels of paradichlorobenzene in blood are attained within a few hours of oral dosing. Studies of rats orally exposed to ^{14}C-labelled paradichlorobenzene indicate the following distributional events after absorption from the gastrointestinal tract: 1) translocation of parent compound to the liver where considerable metabolism occurs; 2) biliary excretion and intestinal re-absorption of metabolites (i.e., enterohepatic recirculation); 3) eventual translocation of most metabolites to the kidney for elimination via the urine; 4) temporary storage of parent compound in fat when metabolism is saturated; and 5) minor distribution of parent compound or metabolites to tissues other than fat, kidney, and liver.

Compared with plasma levels, the concentration of the chemical (and/or metabolites) in the liver and kidneys is *ca* 2.5-fold higher, and the concentrations in adipose tissue are at least *ca* 25 times greater. This is attributable to the lipophilicity of paradichlorobenzene. The disparity between levels in fat and elsewhere becomes even greater when the chemical is inhaled, due to the absence of 'first pass' metabolism within the liver.

The proposed pathway for paradichlorobenzene metabolism is shown in Appendix 1. The first step in the metabolism is CYP P450 catalysed oxidation of the aromatic ring, generating an epoxide. Treatment of male rats and mice with paradichlorobenzene results in a sustained increase in hepatic CYP P450 levels. In humans, metabolism proceeds predominantly via a 2,3-epoxide while in rats and mice, metabolism proceeds via 1,2- and 2,3-epoxides. The epoxides can react directly with DNA and cellular proteins, can react directly or via enzymatic catalysis with GSH to form a GSH conjugate, or can be hydrolysed to 2,5-dichlorophenol (detected in human urine) and minor amounts of 2,4-dichlorophenol. The dichlorophenols can be further oxidized to dichlorocatechols and dichlorohydroquinones, or conjugated with GSH, glucuronic acid or sulfate. Considerable levels of secondary metabolites and only small amounts of dichlorophenols have been detected in the urine of exposed animals, indicating that secondary metabolism is extensive.

Paradichlorobenzene and its metabolites are excreted mainly via the urine, with the faeces and exhaled air being minor routes. Excretion proceeds moderately rapidly, with radioactivity declining to relatively low levels within 24 hours of exposure and being eliminated from tissues within 120–196 hours.

Acute toxicity, irritation and sensitisation

Paradichlorobenzene is of low acute toxicity via the oral, dermal and inhalation routes. In rats, the oral and dermal median lethal dose (LD_{50}) values are >2000 mg/kg bw while the inhalational median lethal concentration (LC_{50}) is >5070 mg/m^3. The chemical is slightly irritating to rabbit eyes and skin, and its crystals and vapour irritate human eyes and mucous membranes within the respiratory tract. Paradichlorobenzene does not sensitise the guinea pig skin when applied percutaneously, but does cause weak sensitisation if guinea pigs are induced intra-dermally. A few clinical case reports suggest that the chemical can induce a systemic allergic response in some persons, but negative results were obtained in a subchronic immunotoxicity study in guinea pigs (including a passive anaphylaxis test).

Toxicity in humans

Despite a prolonged history of industrial use and widespread availability of paradichlorobenzene- based air fresheners and moth repellents, there is only a limited amount of information available on the chemical's toxicity to humans. There are no epidemiological studies on cancer or non-neoplastic diseases in exposed industrial workers or consumers. A handful of clinical case reports on persons ingesting or inhaling paradichlorobenzene suggest that the liver, kidney, nervous system and haematopoietic system are the principal target organs/tissues for systemic toxicity. However, a lack of quantitative exposure data prevents the definition of dose-effect or dose-response relationships, and so these observations cannot be used to derive a TDI for humans.

Toxicity in laboratory animals

The findings on paradichlorobenzene in experimental animals are consistent with those from humans in that the liver, kidney, and respiratory, haematopoietic and nervous systems are the main target organs/tissues. Furthermore, the adrenal, thyroid and parathyroid glands were affected in some studies in rats or mice. Although causing materno- and fetotoxicity and toxicity to neonates in reproduction and developmental studies, paradichlorobenzene did not cause malformations or have effects on fertility or reproductive behaviour.

Haematological effects

Methaemoglobinaemia has occurred in workers exposed to paradichlorobenzene, and there are reports of haemolytic anaemia in other persons exposed to the chemical in domestic settings. Although haemolytic anaemia has not occurred in studies with paradichlorobenzene in experimental animals, a variety of other haematological effects were seen. In the NTP 13-week oral study WBC, neutrophil and lymphocyte counts became depressed in male mice at $\geq$600 mg/kg bw/d and in females receiving $\geq$1000 mg/kg bw/d. The males also displayed reductions in reticulocytes. Lymphoid necrosis in thymus and depletion in the spleen were observed together with haematopoietic hypoplasia of the spleen and bone marrow in premature decedents at 1500 or 1800 mg/kg bw/d. Treatment-related abnormalities were seen in the spleen, lymph nodes and thymus of mice receiving $\geq$300 mg/kg bw/d in the NTP 24-month mouse study.
Haematological effects in rats presented as mild microcytic anaemia (at a lowest dose of 120 ppm or 22 mg/kg bw/d in a 13-week inhalation study [Aiso *et al.*, 2005a]) without any effects on WBCs. Decreased spleen weights and bone marrow hyperplasia occurred at the relatively high dose of 1200 mg/kg bw/day in the NTP
13-week rat study. Chronically treated dogs displayed anaemia, bone marrow hyperplasia and increased splenic haematopoiesis at 75 mg/kg bw/d.

Toxicity to nasal passages and respiratory tract

In chronic exposure inhalation studies performed with paradichlorobenzene on rodents, the most sensitive toxicological end-point was injury to the olfactory epithelium. The nasal lesions were characterised by respiratory metaplasia of the nasal gland epithelium in mice and rats and the olfactory epithelium in mice, and by the loss of olfactory cells and appearance of abnormal eosinophilic globules in the respiratory and olfactory epithelia of female rats. These lesions have been reported to occur in ageing mice and rats of both sexes (Nagano *et al.*, 1997).

Aiso *et al.* regarded the phenomenon as resulting from an acceleration or exacerbation of a spontaneous ageing lesion or degeneration. The physiological significance of 'goblet' formation is uncertain but Aiso *et al.* suggested it was correlated with the accumulation of proteinaceous material in the cells supporting the olfactory and respiratory epithelia.

Given that the effect occurs on the tissue which is directly and heavily exposed to paradichlorobenzene vapour, this toxicological end-point may be regarded as a portal-of-entry effect, possibly mediated by irritation to mucous membranes. It is of low relevance to risk assessment of humans exposed orally by dietary ingestion.

Adrenal toxicity

In the presence of capsular and medullary hyperplasia, benign and malignant adrenal pheochromocytomas developed in male mice treated at 600 mg/kg bw/d during the US NTP (1987) oral carcinogenicity study. No such effect occurred in female mice, or rats. Although tumour incidences were comparatively low (4% and 8% at 300 and 600 mg/kg bw/d, respectively) the historical control range was exceeded at the higher dose, and the NTP appears to have considered that the tumours were related mechanistically to the hyperplasia. However, adrenal tumours were not reported in a 24-month inhalational carcinogenicity study with mice at atmospheric concentrations up to 300 ppm, equivalent to 474 mg/kg bw/d. The adrenal tumours therefore are a high-dose phenomenon restricted to one sex in one species, and merit a low level of concern from a regulatory perspective.

Thyroid toxicity

A positive dose-response trend for follicular adenoma of the thyroid gland was observed in female mice treated at 300 and 600 mg/kg bw/d in the US NTP (1987) oral carcinogenicity study. However, the increased incidence was not statistically significant. paradichlorobenzene also caused thyroidal hyperplasia (but not neoplasia) in male mice. The NTP concluded that this 'marginal' effect may have been biologically significant, given that the related chemical hexachlorobenzene causes alveolar adenomas of the thyroid gland in male hamsters.

It is possible that the hepatic enzyme-inducing effect of paradichlorobenzene may disturb the thyroid-pituitary axis by increasing the metabolism of thyroid hormones but there is no direct evidence of this, as circulating thyroid hormone levels were not measured in either mice or rats.

The increase in hyperplasia of the parathyroid gland noted in male rats during the NTP 24-month carcinogenicity study is probably secondary to renal toxicity. The US NTP attributed this hyperplastic abnormality to a decrease in functional renal mass, a subsequent alteration in serum phosphate and calcium excretion by the kidney, and stimulation of the parathyroid gland to release parathyroid hormone.

Hepatotoxicity

Liver injury has been observed in most of the species in the toxicological database for paradichlorobenzene, including mice, rats, guinea pigs, rabbits and dogs. In the latter, there was a comparatively steep dose-response relationship, with a NOEL of 10 mg/kg bw/d, for hepatocellular hypertrophy and elevated plasma liver enzyme activity at 50 mg/kg bw/d and hepato-biliary dysfunction at 75 mg/kg bw/d.

Paradichlorobenzene caused hepatomegaly and non-neoplastic liver lesions (hepatocyte degeneration and necrosis, cytomegaly and karyomegaly) in male and female mice, together with hepatocellular adenoma and carcinoma. Necrotic liver injury occurred in the NTP subchronic mouse studies at doses of 600 to 1800 mg/kg bw/d, accompanied (in males) by hepatocellular dysfunction seen as increased blood levels of cholesterol and triglycerides. Males proved to be the more sensitive to formation of adenomas than females: adenoma was observed at a LOEL of 300 mg/kg bw/d in the former compared with 600 mg/kg bw/d in the latter. In both sexes, carcinoma formed only at 600 mg/kg bw/d. In addition, hepatoblastomas (a comparatively rare spontaneous tumour) were observed in males at this same dose.

Inhalation exposure to 300 ppm paradichlorobenzene induced histolytic sarcoma in the liver, hepatoblastoma, hepatocellular carcinoma and hepatocellular adenoma in mice in the 24-month study of Aiso *et al*., (2005b). The consistency in results between this and the US NTP (1987) study is striking but not unexpected in view of the similarity of systemic doses that were achieved. Gavage administration of paradichlorobenzene to mice at 300 and 600 mg/kg bw/d would correspond to systemic uptakes of 213 and 426 mg/kg bw/d, respectively, since the chemical's rate of uptake across the mouse GIT is 71% (EU 2004). By comparison, systemic doses in mice exposed at 300 ppm were approximately 474 mg/kg bw/d.

Liver cancers failed to develop in either mice or rats in Loeser and Lichfield's (1983) inhalation study at atmospheric concentrations of up to 500 ppm. However, the male mice yielded no data because they were withdrawn from treatment prematurely, and even the full duration of this study (57 and 76 weeks in mice and rats, respectively) was too short to effectively evaluate carcinogenic effects in either species.

Subchronically dosed rats displayed perturbed serum lipid levels at $\geq$300 mg/kg bw/d, hepatomegaly at and above 900 mg/kg bw/d, and at 1200 mg/kg bw/d or more they also manifested increased serum AP activity and/or histological features of liver injury similar to those seen in mice. Female rats were less sensitive than males. In contrast to mice, rats did not develop benign or malignant liver tumours, even when treated for 24 months at 300 (males) or 600 (females) mg/kg bw/d in the NTP carcinogenicity study.

The mechanism by which paradichlorobenzene induces liver tumours in mice is not completely defined. As concluded by the IARC (1999), available evidence indicates that the mouse liver tumours may form in response to sustained mitogenic stimulation and proliferation of hepatocytes. Some of the data indicate that the cell proliferation may be a threshold response to cytotoxicity, which would be consistent with the results of the NTP (1987) bioassay. Hepatocellular proliferation may also be due in part to a decrease in the rate of apoptosis, based on evidence from mechanistic studies. The fact that paradichlorobenzene is a hepatic enzyme inducer is probably also relevant.

Rodent liver cancers, especially in mice, that are associated with enzyme induction, hepatomegaly, prolonged cytotoxicity, regenerative hyperplasia and/or mitogenicity are considered as being of comparatively low relevance to human risk assessment (Carmichael *et al.*, 1997). Furthermore, the mouse liver tumours observed in the NTP (1987) and Aiso (2005b) studies developed at relatively high doses, and Aiso demonstrated a NOEC for liver cancer formation of 75 ppm (119 mg/kg bw/d). Nevertheless, as noted by IARC (1999), paradichlorobenzene damages and binds weakly to DNA in mouse liver, and so its mechanism of carcinogenesis may be relevant to humans.

This evidence underlay the IARC's evaluation that paradichlorobenzene is *possibly carcinogenic to humans* (i.e., a Group 2B classification).

Renal toxicity

The renal toxicity of paradichlorobenzene was first demonstrated experimentally during the 1950s, when increased organ weight, cloudy swelling of the tubular epithelium and/or cast formation were observed from threshold doses of 188 mg/kg bw/d and 173 ppm in rats exposed repeatedly by gavage and inhalation. Subsequent studies have consistently shown renal toxicity in mice and dogs, together with the development of adenocarcinoma in male rats.

In chronic toxicity studies, dogs and rats proved to be similarly sensitive to renal toxicity, with kidney discolouration, enlargement and epithelial vacuolation of the renal ducts noted in dogs at oral doses of 50 mg/kg bw/d or more (Naylor, 1996). However, the lesion differed from the one occurring in male rats (see below) insofar as it did not involve hyaline droplet deposition or a similar phenomenon. The NOEL for renal effects in dogs was 10 mg/kg bw/d.

Mice proved relatively resistant to renal injury, with nephropathy and tubular regeneration seen at $\geq$300 mg/kg bw/d in the NTP (1987) oral carcinogenicity study, and effects being limited to renal enlargement at 300 ppm (474 mg/kg bw/d) in a 24-month inhalation carcinogenicity study by Aiso *et al.,* (2005b).

Subchronic gavage dosing with paradichlorobenzene resulted in kidney injury to rats, especially in males. In the NTP (1987) 13-week oral study, the consequences of damage were observed grossly as renal enlargement, and histologically in the form of multifocal degeneration or necrosis of the renal cortical tubular epithelial cells at 1200 mg/kg bw/d or more, and tubular degeneration at 600 mg/kg bw/d and above. Tubular regeneration was slightly increased at 300 mg/kg bw/d. Functional impairment occurred in males treated at $\geq$900 mg/kg bw/d, seen as elevations in BUN. Nephropathy, increased urine volume, acidity and protein excretion occurred in male rats at the lowest administered dose of 75 mg/kg bw/d in a second 13-week oral study, by Bomhard *et al.,* (1988). Abnormal renal function tests and nephropathy also developed in male rats treated for 13 weeks by inhalation at $\geq$270 ppm ($\geq$48 mg/kg bw/d) (Aiso *et al.*, 2005a). This latter study demonstrated a NOEL of 120 ppm (22 mg/kg bw/d) for renal toxicity.

In the US NTP (1987) oral carcinogenicity study, paradichlorobenzene caused renal toxicity in male and female rats, but the features and severity differed between the sexes. Nephropathy occurred more frequently in females in response to gavage dosing with a LOEL of 300 mg/kg bw/d. A NOEL for this injury in females was not established. Under the same experimental conditions, the test chemical exacerbated the severity of nephropathy (the incidence was uniformly high, as this is a common ageing lesion in male rats) in males at a LOEL of 150 mg/kg bw/d. At and above this same dose, male rats displayed additional lesions: epithelial hyperplasia of the renal pelvis, mineralisation of renal medulla collecting tubules, and formation of renal tubular cell adenocarcinomas. The study did not demonstrate a NOEL for these effects in males.

In their 24-month rat study by inhalation, Aiso *et al.,* (2005b) found renal enlargement (both sexes) and urothelial hyperplasia and papillary mineralisation (in males only) at 300 ppm.

Their failure to engender α_{2u}-globulin deposition and renal carcinogenicity may be attributed to insufficiently high systemic doses. An atmospheric concentration of 300 ppm would have delivered a systemic dose of only *ca* 54 mg/kg bw/d, compared with an estimated 93 and 186 mg/kg bw/d at the low and high doses in the US NTP gavage study, assuming a GIT absorption rate of 62% in rats (see Assessment). The NOEL for renal toxicity in Aiso's study was 75 ppm (14 mg/kg bw/d).

The renal lesions developing in male rats gavaged with paradichlorobenzene during the NTP 13-week and 24-month studies were similar to those seen in male rats exposed to gasoline vapours, related petroleum naphthas, light hydrocarbons consisting of paraffins, cycloparaffins or alkyl aromatic hydrocarbons, and decalin. Long-term administration of a number of these compounds to male rats produces renal tubular cell adenocarcinoma. Degeneration of epithelium in proximal convoluted tubules, regeneration, and dilated tubules filled with granular proteinaceous material were reported in male rats exposed to light hydrocarbon compounds in short-term inhalation studies. The characteristic 'early' lesion in animals exposed to these hydrocarbons consisted of hyaline droplets in tubular epithelial cells. In rats exposed to decalin, limonene and other hydrocarbons the droplets consisted primarily of lysosomes filled with α_{2u}-globulin (US NTP, 1987).

The protein accumulating within renal tubular cells following treatment with paradichlorobenzene has also been positively identified as α_{2u}-globulin (Tyl and Neeper-Bradley, 1989; Dietrich and Swenberg, 1991), a low-molecular weight protein produced in large quantity by the liver of male Sprague-Dawley and Fischer 344 rats, but not female rats or by any other species (Aiso, 2005a). It is filtered at the glomerulus, partly reabsorbed, and hydrolysed in the cells of the renal P2 segment.

In male rats of susceptible strains, paradichlorobenzene and its major metabolite 2,5-dichlorophenol bind reversibly to α_{2u}-globulin (Charbonneau *et al.*, 1989), causing the protein to accumulate within lysosomes in the cytoplasm of P2 cells. This complex (which resists catabolism by the lysozymes) can lead to an overload of lysosomes, cellular death, increased P2 cell turnover and proliferative activity that favours the formation of adenocarcinomas. However, male NBR rats (which do not synthesise α_{2u}-globulin) do not develop hyaline droplets or renal injury after oral treatment with 500 mg/kg bw/d of paradichlorobenzene, conditions which caused renal injury and hyaline droplet formation in F344 males (Dietrich and Swenberg, 1991). There is now abundant evidence that this process is both sex- and species-specific and is unlikely to be relevant to humans (WHO, 1991; IARC 1999; EU, 2004 and US EPA, 2006). The US EPA (1991) has concluded that 'Male rat renal tubule tumours arising as the result of a process involving α_{2u}-globulin do not contribute to the qualitative weight-of-evidence that a chemical poses a human carcinogenic hazard. Such tumours are not included in dose-response extrapolations for the estimation of human carcinogenic risk.'

Genotoxicity

In vitro studies

Association of [^{14}C]-paradichlorobenzene with calf thymus DNA was detected *in vitro* after incubation with various subcellular fractions of liver and lung, but not of kidney, from rats and mice. However, no adducts have actually been isolated and identified.

Furthermore, no DNA adducts were detected by ^{32}P-post-labelling in calf thymus DNA after incubation with liver microsomes from rat, mouse or humans. paradichlorobenzene was not mutagenic to *S. typhimurium* strains TA100, TA1535, TA1537, TA1538 or TA98 with and without metabolic activation.

Paradichlorobenzene induced mitotic gene conversion and reverse mutation in *Saccharomyces cerevisiae* in the presence of a metabolic activation system from liver of induced mice. It also induced reverse mutations in *Aspergillus nidulans* in the absence of exogenous metabolic activation system. Paradichlorobenzene did not induce DNA fragmentation in cultured rat or human hepatocytes in one study, but in another study using the Comet assay, DNA damage was induced in rat and human kidney cells. It did not induce sister chromatid exchange in Chinese hamster ovary cells in the absence or presence of rat liver S9, but it increased the frequency of sister chromatid exchange in human peripheral blood lymphocytes in the absence of exogenous metabolic activation. Chromosomal aberrations were not induced in *in vitro* studies that used Chinese hamster cell lines and human lymphocytes. However, the frequencies of micronucleated cells were significantly increased in cultured rat hepatocytes and rat and human kidney cells, but not in cultured human hepatocytes.

Paradichlorobenzene did not induce mutations in some studies of the *hprt* locus in hamster cells, while other studies of the *hprt* locus of hamster cells and the *tk* locus of mouse lymphoma cells gave inconclusive results. The frequency of transformation was not increased in treated BALB/3T3 cells.

In vivo studies

After intraperitoneal injection, an association was observed between [^{14}C]-paradichlorobenzene and DNA from liver, kidney, lung or stomach of mice, but not of rats. A ^{32}P-post-labelling technique failed to detect adducts in DNA of liver of rats treated to induce CYP enzymes before IP injection of paradichlorobenzene. Furthermore, oxygen adducts with DNA were not increased in the kidneys of orally dosed rats. Thus, there is no evidence for association or adducts with DNA in rats, whereas there is some evidence for the association (adducts not having been isolated) of paradichlorobenzene with DNA in mice. Significant increases in the frequencies of alkali-labile DNA lesions were detected by the Comet assay in kidney cells from orally dosed rats and in liver and spleen (but not in kidney, lung or bone marrow) of mice dosed IP with paradichlorobenzene. However, unscheduled DNA synthesis was not induced in either male or female rat kidney cells or mouse hepatocytes after single oral doses. S-Phase DNA synthesis was increased in male and female mouse liver and in male (but not female) rat kidney. There was no increase in the frequency of chromosomal abnormalities in bone marrow cells of rats after inhalation exposure to paradichlorobenzene. A dose-dependent increase in the frequency of micronucleated polychromatic erythrocytes was detected in the bone marrow of male mice after IP injection of paradichlorobenzene, but no response was found in other studies in mice after either IP or PO dosing. Moreover, no significant increase in the frequency of micronucleated peripheral blood reticulocytes was seen in male mice given PO or IP doses or in mice given toxic PO doses for 13 weeks. While most evidence does not support an effect of paradichlorobenzene on the frequency of micronucleated cells in maturing erythrocytes, one study did find an increase in the frequency of micronucleated kidney cells from IP-dosed rats. Paradichlorobenzene did not induce dominant lethal mutations in mice.

Overall, the lack of evidence from bacteria, the weak evidence from mammalian cells *in vitro* and the inconsistent findings *in vivo* do not provide convincing evidence that paradichlorobenzene is a classical genotoxin as defined by the accepted, standard tests for genotoxicity. Evidence to the contrary comes mainly from novel or non-standard tests that are not fully recognised by regulatory authorities. Nevertheless, while the positive test results should not be ignored, the overall weight of evidence indicates that paradichlorobenzene does not have significant genotoxic potential. The clear consensus among the IARC, US NTP, WHO, EU and US EPA, together with a German AGS position paper examining the genotoxicity classification of paradichlorobenzene (2003), is that the chemical should not be considered as genotoxic, and that its carcinogenic behaviour in experimental animals arises from mainly epigenetic causes.

Reproductive and developmental toxicity

In the two-generation rat reproduction studies by oral and inhalational administration, there were no treatment-related effects on male or female fertility or mating. However, there was evidence of fetotoxicity, seen as reduced birth weights and survival *in utero*, and rat pups were sensitive to paradichlorobenzene during the pre-weaning period. In addition to poorer survival during the first few days after birth, pups from the oral study (Bornatowicz, 1994) displayed dry and scaly skin and tail constriction, reached some physical developmental landmarks later than controls, and also had impeded neurobehavioural performance. These effects occurred at 90 and/or 270 mg/kg bw/day. Inhalation exposure at 538 ppm (97 mg/kg bw/d) impaired only pre- and post-natal survival (Tyl and Neeper-Bradley, 1989).

Developmental studies (by gavage in rats and inhalation in rabbits) revealed that paradichlorobenzene is fetotoxic at maternally toxic doses, but does not cause malformations. Foetal effects were confined to increased skeletal anomalies and reduced bodyweight in rats (at $\geq$250 and 500 mg/kg bw/d, respectively), and an increased incidence of circulatory system variants in rabbits at 800 ppm (669 mg/kg bw/d).

Relevant dose levels to establish a health standard

To identify the lowest NOELs and NOECs for use in establishing a TDI for paradichlorobenzene, a summary of those determined in studies considered suitable for risk assessment purposes is shown in the following table. It can be seen that when male rat-specific nephropathy and portal-of entry effects on the respiratory tract are discounted, the lowest NOEL demonstrated for toxicological endpoints relevant to human dietary risk assessment is 10 mg/kg bw/d. Accordingly, the TDI for paradichlorobenzene is 0.1 mg/kg bw/d, based on applying a 100-fold safety factor to a NOEL of 10 mg/kg bw/d for renal, hepatic and haematopoietic toxicity and increased adrenal and thyroid weights in a 12-month study in dogs by oral administration (Naylor *et al.*, 1996).

Table A: Summary of Studies Considered

Study	NOEL or NOEC	LOEL or LOEC & effects	Reference
13-wk mouse, gavage	NE	600 mg/kg bw/d Toxicity to liver & haematopoietic system	US NTP (1987)
13-wk mouse, inhalation	120 ppm = 189 mg/kg bw/d	270 ppm = 426 mg/kg bw/d Toxicity to liver	Aiso *et al.,* (2005a)
13-wk rat, gavage	NE	300 mg/kg bw/d Reduced bw gain, toxicity to haematopoietic system, ♂-specific nephropathy	US NTP (1987)
13-wk rat, inhalation	55 ppm = 9.8 mg/kg bw/d	120 ppm = 22 mg/kg bw/d Toxicity to liver & haematopoietic system	Aiso *et al.,* (2005a)
24-mo mouse, gavage	NE	300 mg/kg bw/d Hepatocellular adenoma & toxicity to adrenal, haematopoietic system, kidney	US NTP (1987)
24-mo mouse, inhalation	20 ppm = 32 mg/kg bw/d for portal-of-entry effects 75 ppm = 119 mg/kg bw/d for systemic effects	75 ppm = 119 mg/kg bw/d for portal-of-entry effects 300 ppm = 474 mg/kg bw/d for systemic toxicity (neoplastic hepatic disease, & increased renal weights)	Aiso *et al.,* (2005b)
24-mo rat, gavage	NE	150 mg/kg bw/d [♂] Nephropathy, renal adenocarcinoma, hyperplasia in parathyroid 300 mg/kg bw/d [♀] Nephropathy	US NTP (1987)
24-mo rat, inhalation	20 ppm = 3.6 mg/kg bw/d for portal-of-entry effects 75 ppm = 14 mg/kg bw/d for systemic effects	75 ppm = 14 mg/kg bw/d for portal-of-entry effects 300 ppm = 54 mg/kg bw/d for systemic toxicity (elevated kidney & liver weights, liver cell hypertrophy, renal mineralisation, hyperplasia)	Aiso *et al.,* (2005b)
12-mo dog, oral capsule	10 mg/kg bw/d	50 mg/kg bw/d Toxicity to liver, kidney, haematopoietic system and increased adrenal and thyroid weights	Naylor *et al.,* (1996)
1-gen rat reproduction, dietary admixture	2 mg/kg bw/d for effects on foetuses & pups (the only dose administered)	NE	Makita (2004)
2-gen rat reproduction, gavage	30 mg/kg bw/d for effects on pups 270 mg/kg bw/d for effects on mating & fertility in adults	90 mg/kg bw/d Foetotoxicity, deficits in post-natal development, increased postnatal mortality	Bortanowicz (1994)

Study	NOEL or NOEC	LOEL or LOEC & effects	Reference
2-gen rat reproduction, inhalation	211 ppm = 38 mg/kg bw/d for effects on pups NE in adult males due to male-specific nephropathy at the lowest exposure level of 66 ppm = 12 mg/kg bw/d	598 ppm = 97 mg/kg bw/d Foetotoxicity, increased postnatal mortality In adult females 211 ppm = 38 mg/kg bw/d Increased absolute and relative liver weights	Tyl & Neeper-Bradley (1989)
Rat developmental, gavage	NE for maternotoxicity 250 mg/kg bw/d for foetuses	250 mg/kg bw/d Reduced food consumption 500 mg/kg bw/d Skeletal anomalies	Giavini *et al.,* (1986)
Rat developmental, inhalation	500 ppm = 75 mg/kg bw/d for dams & foetuses	NE No higher doses were administered	Loeser & Litchfield (1983)
Rabbit developmental, inhalation	100 ppm = 84 mg/kg bw/d for does 300 ppm = 251 mg/kg bw/d for foetuses	300 ppm = 251 mg/kg bw/d Bodyweight loss 800 ppm = 669 mg/kg bw/d Circulatory system variations	Hayes *et al.,* (1985)

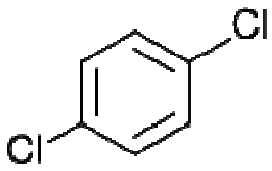
NE = Not established *Discounting male-specific renal nephropathy

PHYSICO-CHEMICAL PROPERTIES

Chemical name: 1,4-dichlorobenzene (CAS)
[This substance is considered by the International Organization for Standardization not to require a common name; 'p-dichlorobenzene' is given as an alternative.]

Alternative names: *p*-dichlorobenzene (IUPAC)
para-dichlorobenzene
paradichlorobenzene
p-DCB
PDB

Chemical structure:



CAS No: 106-46-7
Molecular formula: $C_6H_4Cl_2$
Molecular weight: 147 g/mol
Physical state: colourless solid
Odour: mothball-like
Melting Point: 53 °C
Boiling Point: 174 °C
Vapour Pressure: 0.4 mm Hg at 20 °C; volatile, readily sublimes
Aqueous solubility: 8 mg/100 mL at 20 °C
Octanol/water partition coefficient: Log Kow = 3.44

ASSESSMENT

1 Absorption, Distribution, Metabolism and Excretion

In vitro

den Besten *et al.,* (1992): The oxidative metabolism of 1,4-[^{14}C] dichlorobenzene was studied using liver microsomal preparations from untreated Wistar rats and rats pre-treated with the CYP enzyme-inducers phenobarbitone, 3-methylcholanthrene, isosafrole or dexamethasone. Metabolites formed from the test chemical were analysed and quantified by HPLC/LSC. The extent of covalent binding with microsomal protein was also measured, together with covalent binding to exogenous calf thymus DNA. Molecular orbital computer calculations were used to assist in identifying reactive intermediates and metabolic pathways.

Results

Compared with control microsomes, microsomes from enzyme-induced rats exhibited markedly greater metabolic activity against paradichlorobenzene. This was enhanced 3-fold by phenobarbitone, 5-fold by 3-methylcholanthrene and isosafrole, and 9-fold by dexamethasone pre-treatment, respectively. Protein binding was up to 4-fold more extensive in dexamethasone-induced microsomes than those from rats pre-treated with the other inducing agents. Little or no protein binding occurred in control microsomes.

The major metabolites were 2,5-dichlorophenol and 2,5-dichlorohydroquinone. Smaller quantities of polar metabolites, 3,5-dichlorocatechol and 2,4-dichlorophenol were also detected, as shown in Table 1.1. Formation of polar dihydrodiols was a minor route of biotransformation.

Table 1.1: Metabolism of paradichlorobenzene by dexamethasone-induced rat liver microsomes. Values shown are percentage of total metabolites.

Polar dihydrodiols	2,5-dichlorohydroquinone	3,5-dichlorocatechol	2,4- & 2,5-dichlorophenol*	Covalently bound to protein
4.4-4.6%	24-26%	1.3-4.4%	18-38%	29-50%

*2,4-dichlorophenol accounted for 5 – 10% of the total amount of dichlorophenols detected.

Protein binding was inhibited almost completely by the reducing agent ascorbic acid, with a corresponding increase in the formation of hydroquinones and catechols, indicating the involvement of reactive benzoquinone metabolites in protein binding. Based on the results obtained, the study authors proposed that initially, paradichlorobenzene is oxidised to 1,2- or 2,3-oxides which form (mainly) 2,5-and 2,4-dichlorophenol. These are oxidised to hydroquinone derivatives which in turn form reactive benzoquinones that bind covalently to protein (see metabolic pathway in Appendix 2).

Relative to protein binding, covalent binding to DNA occurred to a small extent. DNA binding in a standard microsomal incubation amounted to levels ‘just slightly above background’ (no data were shown).

In vivo

Hawkins *et al.,* (1980): 1,4-dichloro[U-^{14}C]benzene (radiochemical purity >99% and specific activity 170 µCi/mg) was administered to adult female CFY (Sprague-Dawley derived) rats (bodyweight *ca* 200 g) by inhalation at a nominal concentration of 1000 ppm (6000 mg/m^3) for 3 h/d over 10 days. The test chemical was also given to rats PO or SC at 250 mg/kg bw/d (in 0.5 mL sunflower oil) for up to 10 days. Pairs of rats were sacrificed 24 hours after receiving 2 – 10 doses or exposures and tissues were removed for measurement of radioactivity by LSC. Additional rats were killed at intervals ranging between 5 minutes to 192 hours after the 10th exposure. Tissue radioactivity was quantified in these animals, and in the excreta and exhaled air of two additional rats/group.

Biliary, urinary and faecal excretion were quantified in further animals that received a single 3-hour atmospheric exposure at 1000 ppm or a 250 mg/kg bw PO or SC dose. Metabolites in urine and bile were extracted, purified and identified by TLC, HPLC and GC/MS.

Results

Twenty four hours after repeated daily inhalation, PO and SC dosing, the radioactivity concentrations in tissues of animals dosed orally were lower than those treated via inhalation and SC injection. Generally, the highest levels were observed following the 4th – 6th doses, after which radioactivity tended to decline. The lowest concentrations of radioactivity were present in plasma, muscle and lungs, compared with which levels in the liver and kidney were *ca* twice as high. Levels in fat exceeded those in plasma and other tissues by an order of magnitude.

Table 1.2: ^{14}C concentrations (µg/g or mL) in plasma and tissues 24 hours after the 6th daily dose of 1,4-dichloro[U-^{14}C]benzene (2 rats/treatment)

Matrix	Route of administration		
	Oral	Inhalation	Subcutaneous
Plasma	12	19	25
Liver	14	28	24
Lungs	10	11	14
Kidney	23	43	47
Fat	170	597	269

After the 10th dose, the highest plasma and tissue radioactivity levels occurred during the initial 2 hours but the concentration vs. time course showed some route-dependency. Absorption or distribution of radioactivity subsequent to SC dosing was slowest and peak levels tended to be lower than when the test chemical was administered PO or by inhalation. Depletion from plasma and tissues was also slowest after SC administration. However, with all routes of administration, most ^{14}C residue was lost from plasma and tissues (other than fat) during the first 24–48 hours and radioactivity declined to below the LOD (0.2 µg/g or mL) by 120 hours. Detectable radioactivity levels were still present in fat at the final (192-hour) observation.

During the 120 hours after repeated dosing, 91–97% of administered radioactivity was excreted via the urine, with up to an additional 3% appearing in the faeces.

About 89%, 75% and 41% of this radioactivity was excreted during the initial 24 hours after oral, inhalation and SC dosing, respectively. The slower excretion after SC dosing may reflect the time necessary for the test chemical to disperse from the injection site. The proportion exhaled was 1.0% following PO dosing, 0.2% after inhalation, and 6.4% after SC injection.

In rats with cannulated bile ducts, no ^{14}C was excreted in the faeces during the 24 hours after inhalation or SC dosing, compared with 9% of the administered radioactivity after PO administration. This 9% may represent the unabsorbed portion of the oral dose.

Of the total ^{14}C excreted, 49% was excreted in the bile (vs. 52% in urine) after an inhalation dose, 63% was excreted in bile (vs. 28% in the urine) after a PO dose and 46% and 54% appeared in the bile and urine, respectively, following SC injection. The decreased urinary excretion by cannulated rats (as compared to intact animals) is consistent with enterohepatic recirculation of ^{14}C: much of the material excreted into bile was re-absorbed to be ultimately voided in the urine.

Urine contained 2 major metabolites, a sulfate and a glucuronide conjugate of 2,5-dichlorophenol, representing up to 54% and 34% of urinary ^{14}C, respectively. Two additional minor metabolites were identified by MS as a dihydroxydichlorobenzene (probably 2,5-dichloroquinol) and a mercapturic acid of paradichlorobenzene.

Table 1.3: ^{14}C concentrations (µg/g or mL) in plasma and tissues 1 and 48 hours after the 10th daily dose of 1,4-dichloro[U-^{14}C]benzene (1 rat/observation)

Matrix	Time post-dosing (h)	Route of administration		
		Oral	Inhalation	Subcutaneous
Plasma	1	38	34	42
	48	2	9	22
Liver	1	82	97	35
	48	7	16	22
Lungs	1	43	84	22
	48	3	8	15
Kidney	1	56	304	54
	48	3	15	38
Fat	1	421	2434	335
	48	56	233	424

2,5-Dichlorophenol glucuronide was a major metabolite in bile, accounting for 30 – 42% of biliary radioactivity. A further metabolite (comprising 11, 7 and 42% of ^{14}C in bile following PO, SC and inhalation dosing, respectively) was possibly a sulfur-containing conjugate, but was not characterised further. Two additional metabolites were detected but not identified: one of these was a polar compound accounting for 47, 48 and 12% of ^{14}C in bile following PO, SC and inhalation dosing, respectively. This metabolite was not detected in urine and so must have been further metabolised, either in the intestinal tract or during re-absorption. 2,5-dichlorophenol sulfate was not detected in bile.

The study authors postulated that paradichlorobenzene is metabolised to a 2,3-arene oxide intermediate, which rapidly isomerises to 2,5-dichlorophenol.

Kimura *et al.*, (1979): Paradichlorobenzene was administered PO at 200 or 800 mg/kg bw (in corn oil at a dose volume of 0.5 mL/kg bw) once or daily for 7 days to fasted male Wistar rats (*ca* 200 g bodyweight). Metabolites were extracted from urine, faeces and blood, purified by TLC, and characterised by GC/ECD and/or UV/NMR/MS.

Results

Six hours after administration of a single 200 mg/kg bw dose, a peak blood level of *ca* 17 µg/mL paradichlorobenzene was attained, declining to *ca* 3 µg/mL between 18 and 24 hours, and zero by 48 hours. The concentrations of paradichlorobenzene in heart, brain and lung between 3 and 12 hours post-administration were similar to those in blood. Kidney and liver contained maxima of *ca* 32 and 23 µg paradichlorobenzene/g over the period spanning 6 – 12 hours after dosing. Depletion from kidney, heart and brain was complete within 24 hours, while liver and lung no longer contained residues at 48 hours. By contrast, paradichlorobenzene levels in adipose tissue reached a maximum of 800 µg/g at 12 hours, decreasing to 14 µg/g by the final observation at 120 hours.

'Conjugated' 2,5-dichlorophenol and 3 minor metabolites (free 2,5-dichlorophenol, 2,5-dichlorophenyl methyl sulfoxide and 2,5-dichlorophenyl methyl sulfone) were detected in the excreta. The methyl sulfoxide and sulfone [which may possibly be derived from the mercapturic acid observed by Hawkins *et al*; see above] were also present in the blood, kidney, adipose tissue and liver, within which peak levels of the sulfoxide (respectively *ca* 0.6, 1.8, 0.2 and 0.1 µg/g or mL) were attained 15 hours post-dosing. By 48 hours, the concentration in kidney had declined to *ca* 0.2 µg/g and levels in the blood and remaining tissues were <0.1 µg/g. The time course of the sulfone metabolite's concentration in blood and tissues was characterised by twin peaks at 18 and 48 hours, separated by a transient decline in concentration at 24 hours. Levels in blood, kidney, adipose tissue and liver were *ca* 2.0, 1.0, 1.6 and 0.6 µg/g or mL at 48 hours. Thereafter, the sulfone's half-life in blood and tissues was *ca* 36 hours. Depletion remained incomplete at 120 h, when blood and tissue levels were 0.2–0.3 µg/g or mL. The study authors suggested that 2,5-dichlorophenyl methyl sulfone was a metabolite of the sulfoxide, and may undergo enterohepatic recycling. There was no indication as to whether free or conjugated 2,5-dichlorophenol was detected in the blood or tissues.

Table 1.4: Cumulative urinary and faecal excretion of metabolites by rats following a single oral 200 mg/kg bw dose of paradichlorobenzene

Matrix	Metabolite	% of dose excreted at 24 h	% of dose excreted at 96 h
Urine	Free 2,5-dichlorophenol	2.0	2.3
	Conj 2,5-dichlorophenol	42	46
	2,5-dichlorophenyl methyl sulfoxide	0.013	0.031
	2,5-dichlorophenyl methyl sulfone	0.010	0.122
Faeces	Free 2,5-dichlorophenol	2.7	2.9
	Conj 2,5-dichlorophenol	3.4	3.7
	2,5-dichlorophenyl methyl sulfoxide	0.002	0.003
	2,5-dichlorophenyl methyl sulfone	0.007	0.021

All metabolites were excreted primarily via the urine, with the majority of conjugated and free 2,5-dichlorophenol appearing during the first 24 hours post-dosing.

Urinary excretion of 2,5-dichlorophenyl methyl sulfoxide and 2,5-dichlorophenyl methyl sulfone was most extensive over the first 48–72 hours (see Table 1.4). By 96 hours, 55% dose equivalents had been excreted but the balance was unaccounted for.

The study authors did not comment as to whether the parent chemical was present in the excreta, or identify whether the 'conjugated' 2,5-dichlorophenol was bonded to glucuronyl or sulfate groups.

Loeser and Litchfield (1983): Paradichlorobenzene was administered to rats as a single PO 100 or 1000 mg/kg dose, at 100 or 1000 ppm in the diet for 28 days, or by inhalation over 76 weeks at 75 or 500 ppm followed by a 36-week observation period. In all 3 studies, the parent chemical and its primary metabolite 2,5-dichlorophenol were assayed by GC/ECD after enzymatic cleavage of conjugates in the plasma, urine, adipose tissue, liver and kidneys. In the acute study plasma and tissue residue levels declined rapidly and 40–60% of the dose was eliminated as 2,5-dichlorophenol conjugates in the urine (time interval unspecified).

Although $t_{1/2}$ values were not provided the majority of depletion appears to have taken place over 2–4 days. In the repeat-PO dose study concentrations of paradichlorobenzene in all tissues declined from day 3 to day 7, after which they maintained steady state levels until dosing ceased. For example the concentration in fatty tissue decreased from 48 µg/g on day 3 to *ca* 20 µg/g over days 7-28. One week after treatment ceased, neither the parent chemical or 2,5-dichlorophenol were detectable in any tissue. During inhalation exposure, tissue levels declined between weeks 26 and 76 and residues were not detectable in fat 36 weeks post-exposure. No further data were provided. The study authors considered that the decline in tissue residues during treatment was caused by paradichlorobenzene inducing its own metabolism.

Rabbits

Azouz *et al.*, (1955): Excretion of metabolites was studied in a group of 3 chinchilla rabbits treated with paradichlorobenzene (as a 25% w/v solution in olive oil) via stomach tube at 500 mg/kg bw. Over a 6-day period there was extensive excretion via the urine, which reached its maximum extent 48 hours post-dosing and was still occurring at the end of observation. The major urinary metabolites were a glucuronide conjugate of 2,5-dichlorophenol, sulfated conjugates and 2,5-dichloroquinol, accounting for 36%, 27% and 6% of the dose, respectively. No mercapturic acids or catechols were found in urine. paradichlorobenzene was not detected in the faeces.

Humans

Pagnotto and Walkley (1965): Workers exposed to paradichlorobenzene by inhalation during manufacturing operations excreted 2,5-dichlorophenol in the urine for up to several weeks post-exposure. Levels of the metabolite ranged from 10 to
233 mg/L urine.

2. Acute Toxicity Studies

Table 2.1: Median lethal dose of paradichlorobenzene in laboratory animals

Experimental details	LD_{50} (mg/kg bw)	Major findings	Reference
Rats, PO (No further details)	>2000	No. deaths unstated. Reversible toxic signs were salivation, abnormal gait, hunched posture. Assessed by EU (2004)	Gardner (1987a)
Rats, both sexes (Strain, age & doses unstated); PO	>1000 <4000	No mortality at 1000 mg/kg bw Complete mortality at 4000 mg/kg No. deaths at intermediate doses unstated	Hollingsworth *et al* (1956)
Guinea pigs, both sexes (Strain, age & doses unstated); PO	>1600 <2800	No mortality at 1600 mg/kg bw Complete mortality at 2800 mg/kg No. deaths at intermediate doses unstated	Hollingsworth *et al* (1956)
Rats, dermal (No further details)	>2000	No toxic signs, local effects or macroscopic abnormalities. Assessed by EU (2004)	Gardner (1987b)
Rats, inhalational (No further details)	>5070 mg/m^3	No. deaths unstated. Reversible toxic signs were pulmonary irritation, piloerection, deficit in weight gain. Assessed by EU (2004)	Hardy (1987)
NMRI mice (males only, 8 wk old) Doses not stated; IP	2000	No further information provided.	Mohtashamipur *et al* (1987)
Mice (Strain sex, age & doses not stated); SC	5145	Tremor occurred within 2 – 3 h and continued for > 3 d. No further information provided.	Irie *et al* (1973)

Table 2.2: Irritation and sensitisation of paradichlorobenzene in laboratory animals

Experimental details	Major findings	Reference
Skin irritation, Rabbits (500 mg applied for 4 h as a paste in paraffin oil)	Slight irritation - Erythema, reversible by d 7. Assessed by EU (2004)	Maertins (1988)
Eye irritation, Rabbits (90 mg applied for 24 h as a paste in paraffin oil)	Slight irritation – Conjunctival erythema & oedema, reversible by 72 h. Assessed by EU (2004)	Maertins (1988)
Skin sensitisation, Guinea pigs (Maximisation test, induction at 0.1% ID & 25% dermally. Challenge at 25% dermally, in petrolatum.)	Weak sensitisation. Assessed by EU (2004)	Bornatowicz (1995)
Skin sensitisation, Guinea pigs (Dermal induction at 1 – 30% in paraffin oil)	No sensitisation. Irritation observed during induction. Assessed by EU (2004)	Schmidt (1985a,b)

Allis *et al.,* (1992): Single doses of paradichlorobenzene ranging from 13 to 2790 mg/kg bw were administered by oral gavage (at 10 mL/kg bw dissolved in corn oil at 55 ^{o}C) to 1 Fischer-344 rat/dose level. (Animals were from Charles River Laboratories, Raleigh, NC USA, and were 60 days old). A control animal received vehicle alone.

Twenty-four hours post-dosing, rats were killed by exsanguination under pentobarbital anaesthesia. Bilirubin and cholesterol concentrations and AST, ALT and AP activities were measured in serum. Livers were examined under light microscopy, and CYP 450 levels were measured in excised hepatic tissue.

Results

All rats survived without showing clinical signs. Paradichlorobenzene did not cause hepatic necrosis or biologically significant increases in serum enzyme activities or indicators of hepatobiliary damage, even at the highest dose administered. However, compared with the control value of 3.3%, relative liver weight increased dose-relatedly from 3.5% at 95 mg/kg bw to 4.5% above 2100 mg/kg. Doses of 380 mg/kg bw and higher increased microsomal CYP 450 levels by *ca* 30% (with little or no dose-response), suggesting that paradichlorobenzene may induce its own metabolism in rats. Centrilobular vacuolar degeneration occurred in hepatocytes with a LOEL of 475 mg/kg bw, but there was no dose-response relationship. Severity remained at 'minimal' grade at up to and including the highest dose.

3 Short-term Repeat-dose Studies

14-day gavage studies in mice (US NTP, 1987)

B6C3F1 mice (5/sex/group, 8 wk old, obtained from Harlan Industries, Cumberland, IL USA) were treated for 14 consecutive days with paradichlorobenzene (by oral gavage at 5 mL/kg bw in corn oil) at 250, 500, 1000, 2000 or 4000 mg/kg bw/d (Study 1) or at 60, 125, 250, 500 or 1000 mg/kg bw/d (Study 2). The test chemical (from Dover Chemical Co, NY USA) was of >99% purity and did not contain 1,2- or 1,3-dichlorobenzene at $\geq$ 0.05% w/w, the LOD. In both studies there were 5 control mice/sex. The Study 1 control groups were gavaged with vehicle on day 1 but not treated subsequently. Control mice in Study 2 were left untreated. Animals were housed 5/cage under standard laboratory conditions and provided with feed (Purina Lab Chow, Ralston Purina, St Louis, MO USA) and water *ad libitum*. All animals were necropsied grossly and selected individuals also received a histological examination.

Study 1 results

There was extensive mortality across all groups, but some deaths were accidental. One/5 female and 2/5 male controls were killed by gavage error (probably on day 1). All 4000 mg/kg mice died between days 2 and 4, and there was also complete mortality among 500 mg/kg females. In the remaining groups treated at 2000 mg/kg and below, there were 2 to 4/5 deaths but neither the timing nor incidence of deaths were dose-related. No information about ante-mortem clinical signs was provided. Due to the low survival rates, there were no meaningful data on bodyweight change. No chemical-related effects on gross or histopathology were noted. A NOEL was not demonstrated due to mortality at and above the lowest dose of 250 mg/kg bw/d.

Study 2 results

A single 125 mg/kg male died from gavage-related oesophageal rupture but the test chemical did not cause any deaths, or effects on bodyweight or other parameters. The NOEL was 1000 mg/kg bw/d, the highest dose administered.

14-day gavage studies in rats (US NTP, 1987)

F344/N rats (5/sex/group, 6 wk old, obtained from Harlan Industries, Cumberland, IL USA) were treated for 14 consecutive days with paradichlorobenzene (by oral gavage at 5 mL/kg bw in corn oil) at 60, 125, 250, 500 or 1000 mg/kg/bw/d (Study 1) or at 500, 1000, 2000, 4000 or 8000 mg/kg bw/d (Study 2). The test chemical was the same as administered to mice in the 14-day NTP study (see above). In studies 1 and 2 there were untreated groups of 5 rats/sex, but no vehicle controls. Animals were housed 5/cage under standard laboratory conditions and provided with feed (Purina Lab Chow, Ralston Purina, St Louis, MO USA) and water *ad libitum*. All animals were necropsied grossly. In Study 1 but not Study 2, selected individuals also received a histological examination.

Study 1 results

A single 125 mg/kg male died from gavage-related oesophageal puncture, but the test chemical did not cause any deaths or effects on bodyweight or other parameters. The NOEL was 1000 mg/kg bw/d, the highest dose administered.

Study 2 results

All rats receiving the test chemical at $\geq$ 2000 mg/kg bw/d died after 2 or 3 days' treatment. At 1000 mg/kg bw/d, 1/5 females died from chemical-related toxicity; 1/5 males was killed by gavage error but all others survived. Mortality at 500 mg/kg was confined to a single female on day 13. Terminal bodyweight and bodyweight gain were biologically significantly reduced (by 9 – 14% and 29 – 30%, respectively) in males at 500 and 1000 mg/kg. The terminal bodyweight of the 1000 mg/kg female survivor was depressed by 17% relative to controls. The study authors did not comment on ante-mortem observations, cause(s) of death or effects on gross pathology. Based on the limited information available, there was no NOEL due to the treatment-related female death at 500 mg/kg bw/d, the lowest dose administered.

28-day gavage study in rats (Hollingsworth et al., 1956)

Groups of 2 'young adult white' male rats received paradichlorobenzene at 10, 100 or 500 mg/kg bw/d for 5 d/wk for a total of 20 doses, via stomach tube as a 10% solution in olive oil. There was no control group. Marked cloudy swelling and necrosis in the central areas of the liver lobules and marked cloudy swelling of the renal tubular epithelium with cast formation occurred at 500 mg/kg. At the lower doses the study authors claimed there were no adverse effects (as judged by gross appearance, behaviour, growth, mortality, haematological data and gross and microscopic examination of tissues) and set a NOEL of 100 mg/kg bw/d. However, no supporting data were presented.

16-day inhalation study in various species (Hollingsworth et al., 1956)

Groups of 5 rats, 5 guinea pigs and 1 rabbit/sex were exposed (whole-body) to paradichlorobenzene (purity $\geq$99%) vapour at an atmospheric concentration of 173 ppm (1040 mg/m^3) for 7 h/d, 5 d/wk. Animals received a total of 12 exposures. Similar numbers of control rats and guinea pigs were left unexposed. No adverse effects on growth or survival were reported in either species. In treated rats of both sexes, relative liver weights vs. controls were increased by 19–27% ($p<0.01$), and relative kidney weights were increased by 15-20% ($p<0.01$ in females).

Some treated female rats had very slight congestion and granular degeneration in the central areas of the liver. Guinea pigs displayed a 22–32% decrease in spleen relative weight (without accompanying histological abnormalities). Slight interstitial oedema and congestion and alveolar haemorrhage and oedema occurred in the lungs of some rats, guinea pigs and the female rabbit. No further information was provided. A NOEC can not be set.

83-day inhalation study in rabbits (Pike, 1944)

Comparatively little information on experimental design and findings was provided, but the study was limited to an examination of ophthalmological effects. Paradichlorobenzene (from The Dow Chemical Co, USA) was administered to rabbits by inhalation at 770– 800 ppm (4600–4800 mg/m^3) for 8 h/d for up to 62 exposures over 83 days. All exposed animals displayed tremors and weakness (but no paralysis). Some rabbits died after 'a few' exposures but 3 animals survived until study termination. Lateral nystagmus was observed in several animals, and 'at least' 2 of them displayed transitory corneal oedema. All rabbits showed oedema of the optic nerve and retina. Veins [presumably within the eyel] became congested and enlarged, while arteries became narrowed. However, there were no ocular haemorrhages or exudates, deposits in the vitreous humour or changes within the lens. Two surviving animals were allowed to recover for 17 days. Tremors ceased and bodyweight increased. Optic nerve, retinal and venous abnormalities were partially or fully reversible and the survivors behaved as though there were no disturbance in their vision.

4 Subchronic Studies

13-week studies in mice (US NTP, 1987)

B6C3F1 mice (10/sex/group, 7 wk old, obtained from Harlan Industries, Cumberland, IL USA) were treated on 5 d/wk for 13 weeks with paradichlorobenzene (by oral gavage at 5 mL/kg bw in corn oil) at 0, 600, 900, 1000, 1500 or 1800 mg/kg bw/d (Study 1) or at 0, 84, 169, 338, 675 or 900 mg/kg/bw/d (Study 2). Apart from dose levels, the same protocols were used in studies 1 and 2. The test chemical was the same as administered to mice in the 14-day NTP study (see above). The control groups received vehicle alone. Animals were housed 5/cage under standard laboratory conditions and provided with feed (Purina Lab Chow, Ralston Purina, St Louis, MO USA) and water *ad libitum*. Seven days before sacrifice, urine was collected for 24 h for analysis as described in Appendix 1.
One day prior to sacrifice, retro-orbital venous blood samples were obtained for measurement of haematology parameters (see Appendix 1). Clinical biochemistry analysis (see Appendix 1) was performed in blood samples drawn at sacrifice by cardiac puncture under pentobarbital anaesthesia. All animals were necropsied grossly. Tissues and organs from all vehicle controls and animals from the 2 highest dose groups received a histological examination (see list in Appendix 1). Liver and gallbladder from 600, 900 and 1000 mg/kg males were also examined.

<u>Study 1 results</u>

There was dose-related mortality in both sexes at 1500 and 1800 mg/kg bw/d, and single deaths in the male 1000 mg/kg and control groups (see Table 4.1). At 0 and 1000 mg/kg the deaths occurred during week 13, whereas the deaths at the highest doses took place from day 1 onwards and were spread throughout the treatment period.

There was no indication that any decedents had been killed by gavage error, but the study authors did not comment on whether there were clinical signs in these animals or survivors. Relative to controls, bodyweight gain was significantly ($p<0.05$) reduced in the treated groups except 1500 and 1800 mg/kg females. Terminal bodyweight was depressed in the treated male and female groups by up to 14 and 10%, respectively. However, especially in females, the absence of any dose-relationship suggests that the finding was fortuitous.

The US NTP did not include results of urinalysis in the publication. In haematology (see Table), there was evidence of interference with leucocyte production. WBC count was reduced by 34 – 50% in treated male groups and 27 – 33% in the 1000 and 1500 mg/kg females. There was concomitant depression in lymphocyte count (by 26–33% in treated males and up to 34% in females at ≥1000 mg/kg) and neutrophil count (by 69–82% in treated males only). Although RBC count was not affected in either sex, treated males displayed a 50–80% decrease in reticulocyte count. There was no evidence of an increase in the number of micronucleated RBC in blood smears from treated mice.

Modest elevations in serum AP activity occurred in the surviving 1800 mg/kg males and 900 mg/kg females. Although dose-response relationships were not evident in either sex (see Table 4.1), the finding may have been related to toxicity to the bone or hepato-biliary system (see below). There was also evidence of abnormal lipid metabolism. Males displayed dose-related elevations of up to 2.7- and 1.8-fold in serum cholesterol and triglyceride concentrations, respectively from 900 and 1500 mg/kg upwards. Total serum protein levels were elevated dose-relatedly in 1500 and 1800 mg/kg males. Serum lipid and protein data from females were not reported. Liver porphyrin levels were increased dose-relatedly by 43-83% in males at ≥1000 mg/kg and by *ca* 50% in treated females, albeit without any dose-response relationship. The study authors considered that these increases were 'relatively slight and of little biologic significance' and did not indicate porphyria. When livers from mice were examined under UV light at necropsy, no positive red fluorescence indicative of porphyria was observed.

Organ weight data were presented only as organ:brain weight ratios. These were statistically significantly decreased for spleen in treated males at all doses, and increased for heart in 1800 mg/kg males, ovary in 1500 mg/kg females, thymus in females at ≥900 mg/kg and liver in both sexes at ≥900 mg/kg. Liver, thymus and spleen enlargement were biologically significant, being correlated with histological abnormalities (see below). Data on absolute organ weights or organ:bodyweight ratios would have assisted in clarifying the biological significance of these observations in the heart and ovary, but the absence of effects on histopathology in these particular organs suggests that the results may not be attributable to toxicity. Although there was significant depression in relative kidney:brain weight ratio in some treated male groups from 600 mg/kg upwards, it was non-dose related and there was an opposite trend in females, attaining statistical significance at 1500 mg/kg.

Centrilobular hepatocellular degeneration was observed microscopically at increased incidences in all groups of treated mice. The incidences were 7/10 and 9/10 for males and females in the 600 mg/kg groups, 10/10 in both sexes at 900–1500 mg/kg, and 5/10 and 6/10 in 1800 mg/kg males and females, respectively. Control data were not presented. The severity of hepatocellular degeneration was dose-related. The lesion was associated with cytomegaly, variation in nuclear shape, karyomegaly and granular amphophilic cytoplasm.

Lymphoid necrosis in the thymus, lymphoid depletion in the spleen and haematopoietic hypoplasia of the spleen and bone marrow were also noted in premature decedents that had received 1500 or 1800 mg/kg. However, no incidence data were presented and the severity of the lesions was not described.

The study did not demonstrate a NOEL, due to hepatotoxicity, haematological abnormalities and decreased spleen:brain weight ratio at and above the lowest dose of 600 mg/kg bw/d.

Table 4.1: Findings in mice gavaged with paradichlorobenzene for 13 weeks

Parameter	Sex	Dose (mg/kg bw/d)					
		0	**600**	**900**	**1000**	**1500**	**1800**
Mortality (n/10)	M	1	0	0	1	3	7
	F	0	0	0	0	5	9
Terminal bw (% of controls)	M	-	86	89	88	89	86
	F	-	90	95	95	99	99
Haematology							
WBCs (X1000/mm^3)	M	6.2	4.1*	3.4**	3.8**	3.1**	3.2*
	F	3.6	4.5	3.5	2.6*	2.4*	NE
Lymphocytes (X1000/mm^3)	M	4.1	3.0	2.3*	3.2*	2.7*	2.8
	F	3.3	4.0	3.1	2.4	2.1	NE
Segmented neutrophils (X1000/mm^3)	M	2.1	0.64*	1.0**	0.62**	0.35**	0.37*
Reticulocyte count (% RBC)	M	1.9	1.3	1.1	1.9	2.4	3.3
Clinical biochemistry							
AP (IU activity/L)	M	33	34	32	26	40	58**
	F	64	83	89*	78	56	NE
Cholesterol (mg/dL)^	M	91	95	160*	151**	179**	244**
Triglycerides (mg/dL)^	M	174	134	176	173	262**	305**
Liver porphyrin (ng/g tissue)	M	150	162	173	215*	247**	275**
	F	135	198	156	190*	193	NE
Total protein (g/dL)^	M	5.7	5.4	5.8	5.9	6.6**	7.1**
Organ:brain weight ratios							
Heart	M	3.6	3.6	3.5	3.5	3.2	2.9**
Kidney	M	6.4	5.7*	5.6*	5.7	6.3	5.5
	F	3.9	3.7	4.0	4.0	4.5**	NE
Liver	M	33	33	41**	44**	55**	58**
	F	23	26	34**	30**	45**	NE
Spleen	M	2.3	1.8**	1.5**	1.6**	1.5**	1.4**
Ovary	F	4.8	6.4	8.5	7.6	11**	NE
Thymus	F	8.5	9.4	12*	11*	13*	NE

NE = Not examined ^=Not examined in females *p<0.05 **p<0.01

Study 2 results

No test chemical-related deaths occurred, but up to 2 mice/group were killed by gavage errors. There were no treatment-related effects on bodyweight gain or terminal bodyweight. Results of urine, haematology and clinical biochemistry analyses and gross pathology examinations were not provided. Histologically, centrilobular to midzonal hepatocytomegaly did not occur among controls or animals receiving
338 mg/kg, but was observed in 8 males and 4 females/10 at 675 mg/kg and 9 males and 10 females/10 at 900 mg/kg. The severity of the lesion was minimal–mild at
675 mg/kg and mild–moderate at 900 mg/kg. Findings from organs or tissues other than the liver were not presented.

The highest dose at which there were no treatment-related effects was 338 mg/kg bw/d. However, Study 2 is of limited value for NOEL setting purposes because of the limited range of experimental observations for which data were included.

13-week inhalation study in mice (Aiso et al., 2005a)

In a study designed in accordance with OECD Test Guideline 413, groups of 10 Crj:BDF_1 mice/sex (6 weeks old, from Charles River Japan Inc, Kanagawa Japan) were exposed to paradichlorobenzene vapour at airborne concentrations of 0, 25, 55, 120, 270 and 600 ppm for 6 h/d, 5 d/wk for 13 weeks. Animals were housed under standard laboratory conditions (with free access to CRF-1 pellet diet; Oriental Yeast Co, Tokyo, Japan) and exposed whole-body in chambers. Animals were observed daily, weighed weekly and underwent a complete necropsy. Terminal blood samples were collected under anaesthesia from fasted mice for assay of haematology (RBC, Hb, HCT, MCV and MCH) and clinical biochemistry parameters (Total protein and cholesterol, AST, ALT and BUN). Tissues from all animals were examined histologically. Kidney tissue was also subjected to immunohistochemical examination for binding of anti-α_{2u}-globulin.

Results

Mean achieved airborne concentrations of the test chemical were 25, 55, 119, 268 and 597 ppm, equal to 150, 331, 715, 1611 and 3588 mg/m^3. Assuming that a mouse has a respiratory minute volume of 1239 mL/kg bw (Guyton, 1947) and that the lung absorption rate for paradichlorobenzene is 59% in mice (EU, 2004), the respective daily systemic doses would have been 40, 88, 189, 426 and 949 mg/kg bw/d.

Neither mortality nor clinical signs occurred in any group. The terminal bodyweights of treated male and female groups were not affected at a statistical level, but the treated male groups gained weight more slowly relative to their control group, albeit without a dose-relationship. The sole haematological anomaly found was a slight but statistically significant ($p<0.05$) elevation in mean MCH in 600 ppm females (15.2 vs. 14.9 pg in controls).

Several clinical chemistry indicators of hepatic dysfunction were perturbed, mainly at 600 ppm (see Table 4.2), although ALT activity was also significantly increased in 270 ppm males. Liver weights were increased to a biologically significant extent at 600 ppm and in 270 ppm females. All mice in the 600 ppm groups and all 270 ppm males displayed centrilobular hepatocellular hypertrophy. Severity was slight at 270 ppm but moderate at 600 ppm, and accompanied by focal hepatocellular necrosis in 2/10 600 ppm males.

Despite statistically significant differences between controls and groups exposed at 270 and 600 ppm, renal weights were not affected to a biologically significant extent. No macroscopic or microscopic renal lesions were found at necropsy. The NOEC in this study was 120 ppm, approximately equivalent to an oral dose of 189 mg/kg bw/d, based on liver injury at and above the next highest exposure level of 270 ppm
(426 mg/kg bw/d).

Table 4.2: Findings in mice inhaling paradichlorobenzene for 13 weeks (N=10)

Parameter	Sex	Airborne concentration (ppm)					
		0	**25**	**55**	**120**	**270**	**600**
Terminal bw (g)	M	31	28	28	30	28	28
	F	21	21	21	22	21	22
Clinical biochemistry							
Total protein (g/dL)	M	5.5	5.7	5.7	5.7	5.7	6.1**
	F	5.7	5.6	5.6	5.6	5.8	6.0*
Total cholesterol (mg/dL)	M	84	78	76	86	88	127**
	F	76	72	75	78	87	112**
AST activity (IU/L)	M	42	48	48	46	50	65**
	F	58	63	64	60	65	75
ALT activity (IU/L)	M	11	13	12	13	17*	29**
	F	14	14	15	14	15	28**
BUN (mg/dL)	M	28	29	32	33	36	36*
Organ weights							
Liver, Absolute (g)	M	1.1	1.1	1.1	1.2	1.2	1.6**
	F	0.89	0.87	0.88	0.95	0.98*	1.3**
Liver, Relative (% of bw)	M	3.6	3.9*	3.9*	3.9*	4.4**	5.8**
	F	4.3	4.2	4.2	4.4	4.6**	5.7**
Kidney, Absolute (g)	M	0.43	0.43	0.42	0.43	0.43	0.44
	F	0.28	0.29	0.28	0.29	0.28	0.31**
Kidney, Relative (% of bw)	M	1.4	1.5	1.5	1.5	1.6**	1.5**
	F	1.3	1.4	1.4	1.4	1.3	1.4

*p<0.05 **p<0.01

13-week gavage studies in rats (US NTP, 1987)

F344/N rats (10/sex/group, 6-7 wk old, obtained from Harlan Industries, Cumberland, IL USA) were treated on 5 d/wk for 13 weeks with paradichlorobenzene (by oral gavage at 5 mL/kg bw in corn oil) at 0, 300, 600, 900, 1200 or 1500 mg/kg bw/d (Study 1) or at 0, 38, 75, 150, 300 or 600 mg/kg/bw/d (Study 2). Apart from dose levels, the same protocols were used in studies 1 and 2. The test chemical was the same as administered to rats in the 14-day NTP study (see above). The control groups received vehicle alone. Animals were housed 5/cage under standard laboratory conditions and provided with feed (Purina Lab Chow, Ralston Purina, St Louis, MO USA) and water *ad libitum*. Seven days before sacrifice, urine was collected for 24 h for analysis as described in Appendix 1. One day prior to sacrifice, retro-orbital venous blood samples were obtained for measurement of haematology parameters (see Appendix 1). Clinical biochemistry analysis (see Appendix 1) was performed in blood samples drawn at sacrifice by cardiac puncture under pentobarbital anaesthesia. All animals were necropsied grossly. Tissues and organs from all vehicle controls and animals from the 3 highest dose groups received a histological examination (see list in Appendix 1). Kidney and lungs from the 600 mg/kg groups and 300 mg/kg males were also examined.

Study 1 results

Tremors, poor motor response and ocular discharge were observed among 1200 mg/kg males and in both sexes at 1500 mg/kg. Survival was compromised at $\geq$900 mg/kg (see Table 4.3). Mortality occurred throughout the treatment period except among 1500 mg/kg males, in which 6/8 deaths took place during week 1. Bodyweight gain was inhibited dose-relatedly, with statistical significance ($p \leq 0.05$ or 0.01) *vs.* controls being attained at $\geq$300 mg/kg in males and 1200 mg/kg in females.

This was reflected in terminal bodyweight, which was biologically significantly depressed in treated male groups and in females receiving ≥1200 mg/kg.

Urinary volume rose to *ca* 140% of control values in males treated at ≥1200 mg/kg. A *ca* 300% increase in urinary uroporphyrin excretion and a 600% increase in urinary coproporphyrin excretion were observed in these same groups. A 1200 mg/kg, females did not excrete enhanced amounts of uroporphyrin but coproporphyrin excretion was increased to 200% of the control value (Table 4.3). Unfortunately, threshold doses for these effects can not be established because no measurements were made in the 300–900 mg/kg dose groups. Hepatic porphyrin levels were not increased significantly in either sex at any dose.

Female rats displayed few disturbances in haematological parameters but the test chemical produced statistically significant decreases in haematocrit in males at all doses (Table 4.3), accompanied by significant but non-dose related decreases in RBC count and haemoglobin level. Also in males alone, 25–100% increases in reticulocyte levels were noted but statistical significance was attained only at 300 and 900 mg/kg. MCV was significantly depressed in males and females at and above 900 and 600 mg/kg, respectively, with a dose-response relationship evident in females.

As shown in Table 4.3, peturbation in circulating lipid concentrations occurred with respect to triglycerides (decreased by up to 60% in treated males) and cholesterol (elevated by up to 60% in 600–1200 mg/kg males and 25% in 900 and 1200 mg/kg females). Modest but statistically significant increases in total protein level were observed in females at 900 and 1200 mg/kg. Slight but statistically significant increases in BUN in 900 and 1200 mg/kg males were accompanied by a similar (albeit non-significant) increase in 1200 mg/kg females.

In both sexes, liver:brain weight ratio was increased by *ca* 20–25% at ≥ 900 mg/kg. Males (but not females) receiving ≥600 mg/kg displayed up to 15% increase in kidney:brain weight ratio. Spleen (in both sexes) and thymus (in males only) relative weights became decreased by up to 15% and 45%, respectively. Threshold doses for statistical significance were 1200 and 1500 mg/kg in spleen and thymus (Table 4.3). Statistically significant decreases of up to 40% in uterus:brain weight ratio were seen in groups treated at 300–1200 mg/kg. However, there was neither a dose-response relationship nor any indication of abnormal uterine histology, so it is conceivable that the finding was not toxicologically significant.

Degeneration and necrosis of hepatocytes, hypoplasia of the bone marrow, lymphoid depletion of the spleen and thymus, epithelial necrosis and villar bridging of the small intestinal mucosa, and epithelial necrosis of the nasal turbinates were observed in the rats receiving 1200 or 1500 mg/kg, but not 900 mg/kg or less. There was an increased incidence of lymphocytic perivasculitis in the lungs of males that received the test chemical at 600 or 900 mg/kg. No incidence / severity data on these abnormalities were provided. Most treated males that survived to scheduled sacrifice had renal lesions characterised by multifocal or degenerative necrosis of the renal cortical tubular epithelial cells, with amorphous eosinophilic material in the lumens of the affected tubules. There was an increase in the number and size of eosinophilic droplets in the cytoplasm of epithelial cells of the proximal convoluted tubules. Some thickening of tubular basement membranes was present. Renal tubular cell degeneration was observed in 9 or 10/10 males receiving 300–1200 mg/kg and in 3/10 of the 1500 mg/kg males. Renal injury did not occur in female rats.

Table 4.3: Findings in rats gavaged with paradichlorobenzene for 13 weeks

Parameter	Sex	Dose (mg/kg bw/d)					
		0	**300**	**600**	**900**	**1200**	**1500**
Mortality (n/10)	M	1	0	0	1	5	8
	F	0	0	0	2	1	9
Terminal bw (% of controls)	M	-	89	84	82	76	68
	F	-	102	97	94	89	80
Urinalysis							
Volume (mL/24 h)	M	5.4	NE	NE	NE	7.4	7.8
	F	4.4	NE	NE	NE	5.3	NE
Urinary coproporphyrin (µg/24h)	M	0.30	NE	NE	NE	1.8**	2.0**
	F	0.19	NE	NE	NE	0.42*	NE
Urinary uroporphyrin (µg/24h)	M	0.63	NE	NE	NE	1.7*	1.9*
	F	0.81	NE	NE	NE	1.2	NE
Haematology							
Haematocrit (%)	M	50	48*	47*	47*	48*	43**
Haemoglobin (g/dL)	M	18	16**	17**	17**	17**	15**
MCV (μm^3)	M	51	50	49	48**	49**	48*
	F	53	53	52**	51**	50**	NE
RBC (X1000/mm^3)	M	10.0	9.5**	9.5**	9.7**	9.8*	8.8**
Reticulocytes (% RBC)	M	1.5	2.8**	2.2	3.7**	1.9	1.9
Clinical biochemistry							
Cholesterol (mg/dL)	M	54	59	72**	80**	86**	NE
	F	71	84	69	89**	89**	NE
Triglycerides (mg/dL)	M	209	121**	158**	86**	114**	NE
	F	117	98	118	107	101	NE
Total protein (g/dL)	F	7.0	7.0	6.9	7.4*	7.6**	NE
BUN (mg/dL)	M	23	25	24	28**	28**	NE
	F	24	23	23	22	29	NE
Organ:brain weight ratios							
Kidney	M	5.4	5.7	5.9*	6.2**	6.0*	5.8
Liver	M	51	49	55	61**	61**	56*
	F	30	33	32	36**	38**	NE
Spleen	M	3.3	3.3	3.1	3.3	3.0	2.8
	F	2.5	2.5	2.3	2.3	2.1**	NE
Thymus	M	1.8	1.6	1.7	1.4	1.5	0.97**
Uterus	F	5.1	2.5**	2.2**	2.8**	2.5**	NE

^Not analysed statistically due to insufficient group size. NE = Not examined - = Not relevant
*p<0.05 **p<0.01

Given that bodyweight gain inhibition, renal injury and haematological abnormalities occurred in male rats at the lowest administered dose (300 mg/kg bw/d), the study did not demonstrate a NOEL.

Study 2 results

Up to 2 rats/group died from gavage error but there were no test chemical-related deaths or effects on bodyweight gain and terminal bodyweight. Renal injury occurred in the 600 mg/kg male group, in which 9/10 animals displayed moderate cortical tubular degeneration. The incidence / severity of tubular degeneration in other groups were as follows: control, 7/10 mild; 150 mg/kg, 5/10 mild–moderate; and 300 mg/kg, 3/10 moderate. According to the study authors the test chemical did not cause any histological effects in females.

Based on the limited information available, it appears that no treatment-related effects occurred at 150 mg/kg bw/d. However, as outcomes of urinalysis, haematology, clinical biochemistry or gross pathology investigations were not presented, this study is unsuitable for NOEL-setting purposes.

13-week gavage study in rats (Bomhard et al., 1988)

In a study of renal toxicity, paradichlorobenzene (Bayer AG, Leverkusen, Germany; 99.9% purity) was administered by gavage to F344 rats (from Charles River Wiga, Sulzfeld, Germany; aged 6–8 wk and weighing an average of 176 [♂] / 114 [♀] g at study commencement). Animals were housed under standard laboratory conditions and received water and Altromin 1324 food (Altromin GmbH, Lage, Germany) *ad libitum*. Groups of 10 animals/sex were treated once daily, 7 d/wk at doses of 0, 75, 150, 300 or 600 mg/kg bw/d in corn oil at a dose volume of 2 mL/kg. Controls received vehicle alone. Five rats/sex/group were killed after 4 weeks' treatment and the remainder were sacrificed after 13 weeks.

A limited range of haematology and clinical biochemistry investigations (using retro-orbital blood samples) and comprehensive urinalysis were conducted on days 3 and 9 and at weeks 4 and 12 in 5 rats/sex/group (parameters examined are listed in Appendix 1). All animals were necropsied grossly. Kidneys were weighed and examined by light and electron microscopy, but other tissues were not submitted to histology.

Results

No treatment-related effects on clinical signs, food consumption or growth were observed. At 300 and 600 mg/kg bw/d, 1/10 male rats were killed by gavage error but there was no other mortality. Water consumption of males increased dose-dependently by 20% at 75 mg/kg bw/d to 40% at 600 mg/kg bw/d, while a 23% elevation occurred in 600 mg/kg bw/d females. In treated males, urinary acidification was observed on days 3 and 9 and at week 12, and increased numbers of epithelial cells were voided in urine from day 9 onwards (no quantitative data provided) together with higher amounts of the enzymes NAG [from lysosomes within proximal tubular cells] and LDH (see Table 4.4). Treated males also excreted more total protein in their urine from week 4 onwards. There were general time- and dose-related upward trends in these parameters. In contrast to the results obtained from males, LDH excretion by treated females showed an inverse dose-response relationship from day 9 onwards. Excretion of AAP [from brush border cells] tended to decrease in both sexes at 600 mg/kg and in 300 mg/kg males. Urinary volume, specific gravity and testosterone excretion were unaffected in either sex.

At the 4- and 13-week necropsies, renal pallor was observed in treated males. Renal enlargement was evident in 300 and 600 mg/kg males at week 4 and had become more severe in these groups by study termination, reaching up to 35% higher than for controls. Furthermore, on week 13, absolute and relative kidney weights were also significantly increased in 150 mg/kg males and 600 mg/kg females See Table 4.4).

There was no histological evidence of renal injury in treated females. By contrast, at 4 weeks, morphological abnormalities at the corticomedullary junction were evident in 150 and 600 mg/kg males. These consisted of sporadically dilated tubules of the outer portion of the medulla, which were filled with cellular detritus. Cortical tubules contained granular, sometimes crystalline structures and hyaline intraluminal droplets in the epithelia.

Some tubules were filled with desquamated epithelia. These features became more pronounced by week 13 at 150–600 mg/kg bw/d, accompanied by single cell necrosis, signs of partial tubular epithelial regeneration and nephropathy in the cortex. At 75 mg/kg, the only change from controls was an increased incidence of hyaline droplets within cortical tubular epithelia.

Given the increased water consumption and presence of renal enlargement, biochemical evidence of renal dysfunction and deposition of hyaline droplets within the cortical tubular epithelia at and above 75 mg/kg bw/d, the lowest dose administered, a NOEL was not established in males. The NOEL for renal effects in females was 300 mg/kg bw/d, based on increased water consumption and renal enlargement at the highest dose. Given that a restricted range of parameters was investigated, the study is of limited value for NOEL-setting purposes.

Table 4.4: Findings in rats gavaged with paradichlorobenzene for 13 weeks

Parameter	Sex	Dose (mg/kg bw/d)				
		0	75	150	300	600
Urinalysis						
Protein excretion (mg/24 h)	M	10	16**	21*	21*	23**
	F	1.5	1.1	1.6	1.7	2.5
NAG excretion (mU/24 h)	M	168	241*	265	267	411**
	F	84	81	83	116	63
AAP excretion (mU/24 h)	M	429	413	413	308	325
	F	75	84	87	124	49*
LDH excretion (mU/24 h)	M	296	981**	1396**	1282	1017
	F	80	42	31	307^	11
Kidney weights						
Absolute (mg)	M	1740	1867	2022**	2165**	2284**
	F	1044	1166*	1200	1163	1306*
Relative (mg/100 g bw)	M	607	607	664**	744**	819**
	F	638	658	681	682	753*

*p<0.05 **p<0.01 ^Anomalous value: Standard Deviations for the 0, 75, 150, 300 and 600 mg/kg female groups are 44, 18, 10, 581 and 12.

17-week gavage study on hepatic porphyria induction in rats (Carlson, 1977) (Assessed by US EPA, 2006)

Groups of 5 female rats (strain not reported) were administered 0, 50, 100, or 200 mg/kg bw/d paradichlorobenzene in corn oil by daily gavage for 30, 60, 90, or 120 days. Study endpoints included absolute liver weight, liver porphyrin content, and urinary excretion of porphyrins, porphobilinogen and δ-aminolevulinic acid. Bodyweight, liver histology, and activity of δ-aminolevulinic acid synthase were not evaluated. Absolute liver weights were significantly ($p<0.05$) increased in the 200 mg/kg bw/day group at days 30 and 60 (approximately 18 and 25% higher than controls, respectively), but not after 90 or 120 days of exposure. The only additional significant increase in liver weight was in the 50 mg/kg group after 120 days. Small (10–24%), but statistically significant increases ($p<0.05$) in liver porphyrin levels occurred at 60 days in the 200 mg/kg group and after 120 days at $\geq$50 mg/kg.

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The toxicological significance of the increased absolute liver weight is unclear due to the small magnitude and transient nature of the effect, and the lack of information on change relative to body weight.

The increases in liver porphyrins were considered to be slight and not toxicologically significant, particularly because urinary excretion of δ-aminolevulinic acid and porphobilinogen were not increased. The available information therefore indicates that there was a low potential for porphyria and that there were no clear adverse effect levels for the hepatic endpoints examined in this study.

27-week gavage study in rats (Hollingsworth et al., 1956)

Groups of 10 'young adult white' female rats received paradichlorobenzene (in olive oil with 5-10% acacia gum) via stomach tube at doses of 0, 19, 188 or 376 mg/kg bw/d, 5 d/wk for a total of 138 doses over 192 days. Controls were treated with vehicle alone. No deaths were reported and cataracts were not formed at any dose. At 376 mg/kg, there was a 'moderate' increase in mean liver weight and 'slight' increases and decreases in the mean weight of the kidneys and spleen, respectively.
It is unclear whether the authors were referring to absolute or relative organ weights. Microscopically, there was slight cirrhosis and focal necrosis of the liver. At 188 mg/kg, a 'slight' increase in mean liver and kidney weight occurred. 'Haematological and bone marrow values' were stated to be within the normal range (it is unclear whether the haematopoietic system was examined at 376 mg/kg). The study authors set the NOEL at 19 mg/kg bw/d. The limited reporting and lack of treated male rats preclude the NOEL being used for risk assessment purposes.

13-week inhalation study in rats (Aiso et al., 2005a)

In a study designed in accordance with OECD Test Guideline 413 (but lacking urinalysis), groups of 10 F344/DuCrj rats/sex (6 weeks old, from Charles River Japan Inc, Kanagawa Japan) were exposed to paradichlorobenzene vapour at airborne concentrations of 0, 25, 55, 120, 270 and 600 ppm for 6 h/d, 5 d/wk for 13 weeks. Animals were housed under standard laboratory conditions (with free access to CRF-1 pellet diet; Oriental Yeast Co, Tokyo, Japan) and exposed whole-body in chambers. Animals were observed daily, weighed weekly and underwent a complete necropsy. Terminal blood samples were collected under anaesthesia from fasted rats for assay of haematology (RBC, Hb, Hct, MCV and MCH) and clinical biochemistry parameters (Total protein and cholesterol, albumin, triglycerides, phospholipids, AP, AST, ALT, creatinine and BUN). Tissues from all animals were examined histologically. Kidney tissue was also subjected to immunohistochemical examination for binding of anti-α_{2u}-globulin.

Results

The mean achieved exposure concentrations were 27, 54, 120, 267 and 602 ppm, equal to 162, 325, 721, 1605 and 3618 mg/m^3 respectively. Assuming that rats have a respiratory minute volume of 254 mL/kg bw (Mauderly *et al.*, 1982) and that the lung absorption rate for paradichlorobenzene is 33% in rats (EU, 2004), the respective daily systemic doses would have been 4.9, 9.8, 22, 48 and 109 mg/kg bw/d.

Neither mortality nor clinical signs were observed in any of the treated groups. During the first week of exposure, 600 ppm rats of both sexes showed a transient *ca* 5% decline in bodyweight relative to controls. Both groups subsequently regained weight, so that there were no significant differences from control at termination. Growth rates of the remaining treated groups were similar to controls.

In haematology, males had significant decreases in RBC and Hb at ≥120 ppm, Hct at ≥270 ppm and MCV and MCH at 600 ppm only. These signs of anaemia were mainly absent from treated females, apart from slightly decreased Hb at 600 ppm. However, no histopathological abnormalities were observed in the haematopoietic system of the treated male or female groups.

Notable clinical biochemistry findings were restricted to the 2 upper exposure levels (see Table 4.5), and were consistent with renal functional impairment (in the form of elevated BUN and creatinine concentrations) and hepatocellular dysfunction (seen as abnormal circulating protein and lipid concentrations). However, Serum AST and ALT activities *declined* slightly but significantly at 270 and/or 600 ppm.

Dose-related enlargement of the liver and kidney occurred in treated animals, but males were more sensitive than females. Treated males displayed increases in absolute and relative liver weights at ≥120 ppm, absolute and relative kidney weights at ≥270 ppm and absolute and relative spleen weights at 600 ppm. In females, statistically and biologically significant increases in liver and kidney weights were confined to ≥270 and 600 ppm, respectively.

No macroscopic lesions were found at necropsy. The study authors did not report any remarkable findings in the spleen, notwithstanding the enlargement of this organ in 600 ppm males. Centrilobular hepatocellular hypertrophy was present in most males and some females at 600 ppm. In the kidney, all males in all groups (but no females) had hyaline droplets in the proximal tubular epithelial cells, which were exacerbated at ≥270 ppm compared with the remaining treated and control groups. The droplets stained positively with anti-α_{2u}-globulin. Further evidence of renal injury (seen as granular casts from the necrotic desquamation of the tubular epithelium, tubular necrosis and cell cytoplasmic abnormalities) was noted in the 270 and 600 ppm males. Treatment-related papillary mineralisation and hyaline casts were observed in the 600 ppm males only.

The NOEC was 55 ppm (approximately equivalent to an oral dose of 9.8 mg/kg bw/d), based on haematological abnormalities and increased liver weights at and above the next highest atmospheric concentration (120 ppm, or 22 mg/kg bw/d).

Table 4.5: Findings in rats inhaling paradichlorobenzene for 13 weeks (N=10)

Parameter	Sex	Airborne concentration (ppm)					
		0	**25**	**55**	**120**	**270**	**600**
Terminal bw (g)	M	318	325	331	327	334	321
	F	175	179	185	176	178	182
Haematology							
RBC (10^6/µL)	M	9.4	9.3	9.4	9.2*	8.9**	8.7**
Hb (g/dL)	M	16.1	16.0	16.1	15.7**	15.3**	14.6**
	F	15.9	16.2	15.7	15.8	16.0	15.3*
Hct (%)	M	47	47	47	46	45**	43**
MCV (fL)	M	50.5	50.5	50.5	50.3	50.6	49.5**
MCH (pg)	M	17.3	17.2	17.2	17.1	17.3	16.8**
Clinical biochemistry							
Total protein (g/dL)	M	6.9	7.0	7.0	7.0	7.1	7.6**
	F	6.6	6.7	6.7	6.7	6.9	7.1**
Albumin (g/dL)	M	4.0	4.0	4.0	4.1	4.1	4.4**
	F	3.8	3.9	3.9	3.8	3.9*	4.0**
Total cholesterol (mg/dL)	M	54	54	57	59	66**	85**
	F	75	73	77	78	78	91**

Parameter	Sex	Airborne concentration (ppm)					
		0	**25**	**55**	**120**	**270**	**600**
Triglycerides (mg/L)	M	97	98	87	86	101	65*
Phospholipids (mg/dL)	M	109	109	111	113	126**	147**
	F	142	137	147	148	148	172**
AST activity (IU/L)	M	74	73	74	75	69	54**
	F	78	68	72	77	72	56**
ALT activity (IU/L)	M	25	25	25	24	21**	16**
	F	26	23	23	28	23	17
AP activity (IU/L)	M	302	293	288	288	276**	234**
	F	204	202	221	203	189	184
BUN (mg/dL)	M	18	17	18	18	19	24**
Creatinine (mg/dL)	M	0.5	0.5	0.5	0.5	0.6	0.7**
Organ weights							
Liver, Absolute (g)	M	7.8	8.0	8.4	8.5*	9.4**	11.2**
	F	4.0	4.1	4.5**	4.1	4.4**	5.4**
Liver, Relative (% of bw)	M	2.4	2.5	2.5	2.6*	2.8**	3.5**
	F	2.3	2.3	2.4	2.3	2.5**	2.9**
Kidney, Absolute (g)	M	1.8	1.9	1.9	1.9	2.1**	2.8**
	F	1.1	1.1	1.1	1.1	1.1	1.3**
Kidney, Relative (% of bw)	M	0.56	0.58	0.58	0.59	0.62**	0.87**
	F	0.62	0.61	0.62	0.62	0.65	0.71**
Spleen, Absolute (g)	M	0.49	0.50	0.53	0.53	0.52	0.55**
Spleen, Relative (% of bw)	M	0.16	0.15	0.16	0.16	0.16	0.17**
Liver (n/10)							
Centrilobular hepatocellular hypertrophy	M	0	0	0	0	3	9**
(slight)	F	0	0	0	0	0	3
Kidney (n/10)							
Hyaline droplets, moderate	M	2	2	0	0	5	4
Hyaline droplets, marked	M	0	0	0	0	5	5
Granular casts	M	0	0	0	0	10**	10**
Tubular cell necrosis, proximal tubule	M	0	0	0	0	10**	10**
Cytoplasmic basophilia	M	0	2	3	3	10**	10**
Mineralisation, papilla	M	0	0	0	0	1	7**
	F	1	1	0	1	1	1
Hyaline casts	M	0	0	0	0	0	2

*p<0.05 **p<0.01

Inhalation studies in various species (Hollingsworth et al., 1956)

Groups of rats and rabbits were exposed (whole body) for 8 h/d, 5 d/wk to paradichlorobenzene (purity ≥99%) at an atmospheric concentration of 798 ppm (4800 mg/m^3). Nineteen male rats were exposed 1-46 times; 15 female rats were exposed 9-69 times; 16 male and 7 female guinea pigs were exposed 1–23 or 11–20 times, respectively; and 8 rabbits/sex underwent up to 62 exposures. Two of the rabbits that survived to termination were allowed a 17-day recovery period. An unspecified number of animals were sacrificed intercurrently for histological examination. The study authors did not comment explicitly on whether control animals were included in the experiment, but there is no evidence that they were. Exposed animals appeared unkempt and displayed eye irritation, tremors, weakness, unconsciousness and bodyweight loss. One female and three male rabbits, two male guinea pigs and 2 rats/sex died, but it is unclear how many exposures had been administered to these decedents. An unspecified number were moribund when killed. Reversible, non-specific 'eye ground changes' occurred in rabbits, but there were no effects on the lens or vitreous humour. At necropsy, the livers of exposed animals displayed cloudy swelling and centrilobular necrosis.

Two rabbits had slight pulmonary congestion and emphysema, while slight cloudy swelling of the renal tubular epithelium was observed in female rats. No comment was made on the time course of pathological changes, and no further data were reported.

Twenty male rats and 8 guinea pigs/sex were exposed to paradichlorobenzene vapour for 7 h/d, 5 d/wk for 26 weeks at 341 ppm (2050 mg/m^3). Findings comprised 'slight' growth depression and hepatocellular cloudy swelling, fatty degeneration and focal necrosis in male guinea pigs; and increases in mean liver and kidney weights in male rats (apparently without accompanying histological abnormalities). This experiment appears not to have included control groups.

Groups of 10 rats/sex, 8 guinea pigs/sex and 1 female monkey/exposure level (species not given) were exposed to paradichlorobenzene vapour for 7 h/d, 5 d/wk at 158 or 96 ppm (950 or 580 mg/m^3). Ten male mice were treated at 158 ppm and 10 female mice were treated at 96 ppm. Rats were exposed 126–138 times over 26–28 weeks. The 158 ppm guinea pigs were exposed 110 times over 22 weeks but 96 ppm guinea pigs underwent 138-140 exposures over 28 weeks. Unlike the two experiments described above, control groups of 7-13 rats and 7-8 guinea pigs/sex were 'air exposed' for the same number of days as the treated animals. There is no evidence for control mice or monkeys having been included. For rats and guinea pigs but not other species, data on terminal bodyweight and relative lung, heart, liver, kidney, spleen and testis weights were presented. More detailed analyses were carried out on some animals exposed at 96 ppm, comprising 'haematology data on female rats and on rabbits, qualitative urine tests on female animals of all species for blood, sugar, albumin and sediment, terminal BUN values on female guinea pigs and on rabbits.'

The study authors claimed that there were no adverse effects on survival or growth of rats, mice, rabbits or monkeys at either exposure level. Among guinea pigs there were no deaths, but at 158 ppm terminal bodyweight was depressed in males and females by *ca* 10% relative to controls. At this same concentration there was a significant ($p<0.01$ or <0.05) increase of *ca* 10% in relative liver and kidney weights in rats (both sexes) and female guinea pigs. At 96 ppm relative kidney weight was elevated by *ca* 10% in male rats but the difference from controls was not statistically significant. There was a similar increase in relative liver weight in 96 ppm female guinea pigs. However, interpretation of the findings is complicated by the fact that 'a very slight infectious hepatitis was observed on microscopic examination' of liver from an unstated number of these females.

According to the study authors, microscopic examination of the liver from 158 ppm rats disclosed cloudy swelling and granular degeneration 'of questionable significance' in the parenchymal cells in the central zones. Otherwise, there were no remarkable findings. The study authors set the NOEC at 96 ppm but the presence of hepatitis in guinea pigs and the numerous limitations in the study's design and reporting preclude it from being used for risk assessment purposes.

5 Chronic / Carcinogenicity Studies

24-month gavage study in mice (US NTP, 1987)

B6C3F1 mice (50/sex/group, 8 wk old, obtained from Charles River Breeding Laboratories, Portage, IL USA) were treated on 5 d/wk for 103 weeks with paradichlorobenzene (by oral gavage at 5 mL/kg bw in corn oil) at 0, 300 or 600 mg/kg bw/d.

The test chemical was the same as administered to mice in the 14-day and 13-week NTP studies (see above). The control groups received vehicle alone. Animals were housed 5/cage under standard laboratory conditions and provided with feed (NIH 07 Rat and Mouse Ration; Zeigler Bros., Gardners, PA USA) and water *ad libitum*. No urine, haematology or clinical chemistry analyses were performed. At termination, all animals were necropsied grossly. Tissues and organs from all animals received a histological examination (see list in Appendix 1).

Results

There were no treatment-related effects on survival, growth or bodyweight of mice.

The principal target organs of paradichlorobenzene were the liver, adrenal gland, thyroid, haematopoietic system and kidney. There was a dose-related increase in the combined incidence of all types of adrenal pheochromocytoma in males, which was statistically significant at the high dose (HD) (see Table 5.1). Additionally, hyperplasia of the adrenal capsule and medulla was seen *ca* twice as commonly among low dose (LD) and HD males as among concurrent controls.

Treatment-related abnormalities were present at several sites within the haematopoietic system (lymphoid hyperplasia and angiectasis in lymph nodes at the LD and/or HD; focal hyperplasia in splenic follicles at the LD and HD; lymphoid depletion in the thymus of HD males). However, the test chemical did not have any effect on the incidence of lymphoma or other malignancies within the haematopoietic system.

Increased incidences of renal nephropathy were observed in treated males and, to a lesser extent, females. The nephropathy consisted primarily of degeneration of the cortical tubular epithelium with thickening of the tubular and glomerular basement membranes and increased interstitial collagen. In treated females (but not males) there was also a dose-related increase in the incidence of renal tubular regeneration.

Table 5.1: Findings in mice gavaged with paradichlorobenzene for 24 months

Parameter	**Sex**	**Dose (mg/kg bw/d)**			
		Historical Control Mean (Range)	**0**	**300**	**600**
Mortality (n/50)					
Accidental deaths	M	ND	3	8	9
	F	ND	3	4	3
Intercurrent deaths	M	ND	19	10	11
	F	ND	12	10	12
Terminal deaths	M	ND	28	32	30
	F	ND	35	36	35
Adrenal gland					
Capsule –focal hyperplasia (n/50)	M	ND	11	21	28
Medulla –hyperplasia (n/50)	M	ND	2	4	4
Pheochromocytoma (overall %)	M	2%	0%	4%	8%*
Bile duct –focal hyperplasia (n/50)	M	ND	0	1	5
Gall bladder –epithelial hyperplasia (n/50)	M	ND	1	1	4

Parameter	Sex	Dose (mg/kg bw/d)			
		Historical Control Mean (Range)	0	300	600
Haematopoietic system					
Splenic follicles –focal hyperplasia (n/50)	M	ND	1	4	5
	F	ND	2	2	10
Mandibular lymph node –lymphoid hyperplasia (n/50)	M	ND	1	12	10
	F	ND	3	8	10
Mesenteric lymph node –angiectasis (n/50)	M	ND	8	6	18
	F	ND	0	2	5
Thymus –lymphoid depletion (n/50)	M	ND	1	1	6
Kidney					
Nephropathy (n/50)	M	ND	6	12	15
	F	ND	0	3	3
Tubular regeneration (n/50)	F	ND	4	7	13
Liver					
Hepatocellular degeneration (n/50)	M	ND	0	36	39
	F	ND	0	8	36
Cell size alteration (n/50)	M	ND	0	38	40
	F	ND	0	4	27
Focal necrosis (n/50)	M	ND	1	35	37
	F	ND	1	4	30
Hepatocellular adenoma (overall %)	M	13% (0-28%)	10%	27%*	32%*
	F	3.8% (0-10%)	20%	13%	42%*
Hepatocellular carcinoma (overall %)	M	22% (10-38%)	28%	22%	64%***
	F	3.1% (0-8.0%)	10%	10%	38%***
Hepatoblastoma (overall %)	M	0%	0%	0%	8%
Thyroid					
Follicular cell hyperplasia (n/50)	M	ND	1	4	10
	F	ND	8	5	9
Follicular cell adenoma (overall %)	M	ND	2%	2%	6%
	F	4% (0-10%)	0%	0%	7%

*P<0.05 **P<0.01 ***P<0.001 (Incidental tumour statistical tests) ND = No data

In the liver, the incidences of hepatocellular degeneration with individual cell necrosis, cytomegaly and karyomegaly were moderately to markedly increased in LD and HD mice. Males appeared to be more sensitive to liver injury than females, and the incidences of these features were elevated similarly at both doses. By contrast, a dose-response relationship was clearly evident in females. The primary degenerative abnormality was cellular swelling with clearing or vacuolation of the cytoplasm. Individual hepatocytes had pyknotic or karyorrhetic nuclei and condensed eosinophilic cytoplasm. Some necrotic hepatocytes formed globular eosinophilic masses in the sinusoids. The incidence of hepatocellular adenoma was increased significantly among LD males and HD mice of both sexes, while hepatocellular carcinomas were increased at the HD only. Four hepatocellular carcinoma-bearing HD males also had hepatoblastoma. Although statistically insignificant, the finding was considered biologically significant because this tumour type had not been observed among 1091 corn oil vehicle historical controls.

In males, but not females, there was a dose-related increase in the incidence of thyroid follicular cell hyperplasia. Follicular cell adenoma occurred with a significant (p=0.038) positive trend in females, but the incidence in the HD group was not statistically significantly elevated *vs.* controls and lay within the historical control range. An increased incidence of follicular cell adenoma in HD males was also non-significant statistically and biologically.

The study authors concluded that there was *clear evidence of carcinogenicity* in mice, as shown by increased incidences of hepatocellular adenoma and carcinoma (in both sexes) and adrenal pheochromocytoma (in males). There was no NOEL, due to non-neoplastic histological abnormalities within the adrenal gland, mandibular lymph node, kidney and liver, together with hepatocellular adenoma in mice treated at
300 mg/kg bw/d and above.

13-month inhalation toxicity study in mice (Loeser and Litchfield, 1983)

Groups of 75 Swiss mice of both sexes were exposed by inhalation to paradichlorobenzene vapour at 0, 75 or 500 ppm for 5 h/d, 5 d/wk in steel chambers, for a total of 57 weeks. Survivors were allowed a treatment-free recovery period of up to 18-19 weeks. Clinical condition was recorded regularly. Mice found dead were subjected to gross pathology whenever possible. Detailed macropathology was performed on all mice killed moribund or terminally. Females that had received their treatment for at least 52 weeks underwent histopathological examination (tissues/organs examined histologically are given in Appendix 1).

Results

Due to fighting among the males during the early part of the study, and also the probable occurrence of a respiratory infection, the male groups were terminated after week 57, when *ca* 80% mortality had been approached. No results from males were reported. The target atmospheric concentrations were equal to 450 and 3005 mg/m^3. Assuming that a mouse has a respiratory minute volume of 1239 mL/kg bw (Guyton, 1947) and that the lung absorption rate for paradichlorobenzene in mice is 59% (EU, 2004), the systemic doses would have been 98 and 656 mg/kg bw/d at 75 and 500 ppm, respectively.

There was no treatment-related effect on female mortality. The study authors reported that interpretation of findings in the respiratory tract were difficult to assess because of a high background incidence of respiratory disease. However, it was claimed that there were no treatment-related non-neoplastic effects in these or other tissues examined. The only respiratory tract tumours seen were an osteosarcoma in the nasal sinus of a 75 ppm animal and lung adenomas, present in 3/48, 3/47 and 2/50 mice from the 0, 75 and 500 ppm groups, respectively. The test chemical did not cause neoplasia in the remaining organs and tissues, either.

Prima facie, the NOEC was 500 ppm. However, the study is not considered to be reliable enough to use for risk assessment purposes, especially for assessment of potential carcinogenicity. This is due to the withdrawal of males from treatment, the comparatively short duration of treatment (13 months as opposed to the 24 months now required for carcinogenicity studies under OECD and US EPA guidelines), and the limited reporting of results.

24-month inhalation study in mice (Aiso at al., 2005b)

In a study performed according to OECD Test Guideline 453 and GLP requirements, groups of 50 male and female Crj:BDF$_1$ mice (from Charles River Japan, 6 wk old, bw not stated, fed CRF-1 pellet diet [Oriental yeast Co, Tokyo Japan] and sterile water *ad libitum*) were exposed by inhalation to vapours of paradichlorobenzene (Wako Pure Chemical Industries Ltd, Tokyo Japan; purity $\geq$99.9%) at nominal concentrations of 0, 20, 75 or 300 ppm for 6 h/d, 5 d/wk for 104 wk. Control groups inhaled air only.

Animals were caged individually within steel exposure chambers throughout the entire exposure period. No urinalysis, haematology or clinical chemistry investigations were performed. All animals were necropsied, during which organs were weighed and examined macro- and microscopically. The organs examined were not identified but included the liver, kidney and transverse sections of the nasal passage.

Results

The mean achieved exposure concentrations were 19.9, 74.8 and 298 ppm, equivalent to 120, 450 and 1793 mg/m^3. Assuming that a mouse has a respiratory minute volume of 1239 mL/kg bw (Guyton, 1947) and that the lung absorption rate for paradichlorobenzene is 59% in mice (EU, 2004), the respective daily systemic doses would have been 32, 119 and 474 mg/kg bw/d.

The survival rate of treated females was not affected by treatment but survival rates of all treated male groups were apparently decreased (see Table 5.2). However, only the survival rate of the 300 ppm males was significantly decreased (Kaplan-Meier survival analysis, p value unstated). Of the 19 deaths in this group, 12 were caused by liver tumours, while there were 3 other tumour deaths and 4 deaths from non-neoplastic causes. By comparison, there were 3 liver tumour deaths, 3 deaths from other tumours and 4 non-neoplastic deaths in the male control group. Therefore, the decreased survival of 300 ppm males was attributed to an increase in the incidence of mortality caused by liver tumours.

Growth rate was unaffected except among 300 ppm males, which had a 12% bodyweight decrement at termination compared with controls. Food consumption was unaffected. At 300 ppm, absolute and relative liver weights were significantly elevated in both sexes, and absolute and relative kidney weights were significantly increased in females. Males from this group displayed a statistically non-significant increase in absolute kidney weight but a significant increase in relative kidney weight (see Table 5.2).

An increased incidence of mice bearing macroscopically-observable liver nodules at 300 ppm was reported, but no data were provided. Although there were no histological abnormalities suggesting hepatocellular injury, hepatocellular hypertrophy was observed in most 300 ppm males, together with a significantly elevated incidence of histiocytic sarcoma in the liver. Hepatocellular adenoma was significantly more common within the 300 ppm female group, while both sexes displayed elevated incidences of hepatocellular carcinoma and hepatoblastoma at this same atmospheric concentration.

Table 5.2: Findings in mice inhaling paradichlorobenzene vapour for 24 months

Parameter	Sex	Atmospheric Concentration (ppm)			
		0	**20**	**75**	**300**
Mortality (n/49 or 50)	M	10	18	18	19
	F	22	25	26	24
Terminal bodyweight (g)	M	43	40	42	38**
	F	32	34	31	30
Organ weights					
Liver, Absolute (g)	M	1.7	1.9	2.0	3.2**
	F	1.6	1.9	1.6	5.4**
Liver, Relative (% of bw)	M	4.2	5.1	4.9	8.6**
	F	5.0	5.6	5.0	18**
Kidney, Absolute (g)	M	0.67	0.64	0.66	0.72
	F	0.47	0.47	0.46	0.51*

Parameter	Sex	Atmospheric Concentration (ppm)			
		0	20	75	300
Kidney, Relative (% of bw)	M	1.7	1.7	1.6	2.0**
	F	1.5	1.4	1.5	1.7**
Liver histology (n/49 or 50)					
Hepatocellular hypertrophy;	M	0	0	0	34^^
centrilobular	F	0	0	0	2
Hepatocellular adenoma (overall %)	M	26%	18%	14%	26%
	F	4%	20%	12%	40% ## ~~
Hepatocellular carcinoma (overall %)	M	24%	34%	32%	76% ##~
	F	4%	8%	4%	82% ##~~
Hepatoblastoma (overall %)	M	0	4%	0	16% ##
	F	0	0	0	12% #
Histiocytic sarcoma (overall %)	M	0	6%	2%	12% #~
	F	4%	2%	2%	0
Nasal cavity histology (n/49 or 50)					
Respiratory metaplasia:					
Nasal gland	M	37	42	47^	41
	F	9	6	8	19
Olfactory epithelium	M	23	30	38^^	24
	F	7	6	2	20^^
Lung histology					
Bronchiolo-alveolar carcinoma (overall %)	F	2%	2%	2%	8%

*P≤0.05; **P≤0.01 (Dunnett's Test); ^p≤0.05; ^^p≤0.01 (Chi-square Test); #p≤0.05; ##p≤0.01 (Fisher's Exact Test); ~p≤0.05; ~~p≤0.01 (Peto Test)

In the nasal cavity, statistically and biologically significant increases in the incidence of respiratory metaplasia occurred in males at 75 ppm and in females at 300 ppm. The abnormality was characterised by depletion of olfactory cells and replacement of the olfactory epithelium with ciliated cells similar to those normally present in the respiratory epithelium. At 300 ppm, females displayed a 4-fold increase in the incidence of bronchiolo-alveolar carcinoma relative to other groups (Table 5.2). However, although exceeding the study laboratory historical control mean, the incidence did not exceed the historical control range (2.9% and 8%, respectively), and so the finding is not considered as being treatment-related.

Based on increased incidences of respiratory metaplasia in the nasal gland and olfactory epithelium at and above 75 ppm, the NOEC was 20 ppm, approximately equivalent to an oral dose of 32 mg/kg bw/d. If the respiratory metaplasia is regarded as being a portal-of-entry effect occurring in response to localised irritation, the NOEL for systemic toxicity was 75 ppm (119 mg/kg bw/d), based on increased kidney weights and hepatic neoplasms at 300 ppm (474 mg/kg bw/d).

24-month gavage study in rats (US NTP, 1987)

F344/N rats (50/sex/group, 8 wk old, obtained from Charles River Breeding Laboratories, Portage, IL USA) were treated on 5 d/wk for 103 weeks with paradichlorobenzene (by oral gavage at 5 mL/kg bw in corn oil) at 0, 150 or 300 mg/kg bw/d (males) or 0, 300 or 600 mg/kg bw/d (females). The test chemical was the same as administered to rats in the 14-day and 13-week NTP studies (see above). The control groups received vehicle alone. Animals were housed 5/cage under standard laboratory conditions and provided with feed (NIH 07 Rat and Mouse Ration; Zeigler Bros., Gardners, PA USA) and water *ad libitum*. No urine, haematology or clinical chemistry analyses were performed.

At termination, all animals were necropsied grossly. Tissues and organs from all animals received a histological examination (see list in Appendix 1).

Results

Survival of the 300 mg/kg male group was impaired from *ca* wk 70 onwards, achieving statistical significance ($p<0.01$) *vs.* controls after wk 97 (see Table 5.3). Mortality within the 600 mg/kg female group was slightly but consistently higher than among controls from wk 60 onwards, but statistical significance was not attained. Mean bodyweight of 300 mg/kg males was generally 5–8% lower than controls after wk 38, while a similar degree of bodyweight depression occurred in the 600 mg/kg female group after wk 55.

Mononuclear cell leukaemia in male (but not female) rats occurred with a positive dose-related trend ($p<0.05$). Its incidence in the 300 mg/kg group was significantly greater than among concurrent controls (see Table 5.3) and also exceeded the historical control mean. However, the incidence lay just within the upper limit of the historical control range and treatment did not appear to hasten onset of the disease. Given the malignancy's relatively high incidence among untreated male rats, the NTP concluded that it was 'doubtful' whether the increased incidence of mononuclear cell leukaemia was treatment-related.

The rat kidney was a major target organ of paradichlorobenzene, but the severity and features of renal injury varied between the sexes. The incidence of renal nephropathy, a common age-associated lesion in rats, increased dose-relatedly in treated females. Although its incidence in males did not rise with increasing dose, severity was greater in treated animals than in controls. The lesion was characterised by degeneration and regeneration of the tubular epithelium, tubular dilatation with attenuation and atrophy of the epithelium, granular casts in tubules, thickening of basement membranes and minimal accumulation of interstitial collagen. The test chemical also increased the incidences of mineralisation of the collecting tubules by ten-fold in treated males and two-fold in 600 mg/kg females, with severity increasing in males from mild–moderate in 150 mg/kg to moderate-severe at 300 mg/kg. Minimal–mild hyperplasia of the pelvic urothelium overlaying the renal papillae developed in most treated males and 2/50 of the 600 mg/kg female group, together with focal hyperplasia in the tubular epithelium in 300 mg/kg males.

Renal tubular cell adenocarcinoma developed in some males receiving 150 and 300 mg/kg (reaching statistical significance at the high dose; see Table 5.3). There was a significant ($p<0.05$) positive trend in incidence, and a reduction in the time to first observation from 104 in the control group to 46 weeks at 300 mg/kg. There was also a single case of tubular adenoma in the 300 mg/kg male group. No renal tumours were observed in females.

Table 5.3: Findings in rats gavaged with paradichlorobenzene for 24 months

Parameter	Sex	Dose (mg/kg bw/d)				
		Historical Control Mean (Range)	0	150	300	600
Mortality (n/50)						
Accidental deaths	M	ND	7	5	4	-
	F	ND	0	-	1	3
Intercurrent deaths	M	ND	11	14	26	-
	F	ND	15	-	10	18
Terminal deaths	M	ND	32	31	20	-
	F	ND	35	-	39	29
Terminal bodyweight (g)	M	ND	461	450	424	-
	F	ND	322	-	315	298
Haematopoietic system						
Mononuclear cell leukaemia (Overall %)	M	14% (1-28%)	10%	14%	22%^	-
Kidney						
Nephropathy (n/50)	M	ND	42	42	46	-
	F	ND	21	-	32	41
Cortex –cysts (n/50)	M	ND	0	1	7	-
	F	ND	0	-	1	1
Medulla –mineralisation (n/50)	M	ND	4	46	47	-
	F	ND	5	-	1	10
Pelvis –epithelial hyperplasia (n/50)	M	ND	1	30	31	-
	F	ND	0	-	0	2
Tubule –focal hyperplasia (n/50)	M	ND	0	1	9	-
-adenoma (overall %)	M	0.5% mean for	0%	0%	2%	-
-adenocarcinoma (overall %)	M	both tumours combined	2%	6%	14%*	-
Mesothelioma (n/50)	M	4% (0-12%)	2%	0%	8%	-
Parathyroid (n/38 - 42)						
Hyperplasia	M	ND	4	13	20	-

*P<0.05 **P<0.01 ***P<0.001 (Incidental tumour statistical tests)
^p<0.05 (Life table statistical tests) ND = No data - = Not tested

Mesotheliomas in male (but not female) rats occurred with a significant positive dose-related trend in incidence ($p<0.05$) that exceeded the historical control mean (Table 5.3), but lay within the historical control range and failed to attain statistical significance in direct comparison with concurrent controls. Occurrence of the tumour was therefore considered to be unrelated to treatment. The test chemical caused a dose-related increase in the incidence of hyperplasia within the parathyroid in males only, without accompanying malignancy, or histological abnormalities in the thyroid.

There was no NOEL in males, due to non-neoplastic renal toxicity, development of renal tubular cell adenocarcinoma, and hyperplasia within the parathyroid at and above the lowest dose of 150 mg/kg bw/d. There was also no NOEL in females, due to increases in renal nephropathy at and above the lowest dose of 300 mg/kg bw/d.

18-month inhalation toxicity study in rats (Loeser and Litchfield, 1983)

Groups of 76–79 rats (Alderley Park Wistar-derived strain) of both sexes were exposed by inhalation to paradichlorobenzene vapour at 0, 75 or 500 ppm for 5 h/d, 5 d/wk in steel chambers, for a total of 76 weeks. Survivors were allowed a treatment-free recovery period of up to 36 weeks.

Clinical condition, bodyweights and food and water consumption were recorded regularly. Clinical biochemistry (blood urea and glucose and plasma ALT and AST activities), 'standard' (but unspecified) haematology observations and urinalysis (parameters unstated but including coproporphyrin excretion) were performed on 5 rats/sex/dose at wk 5, 14, 26-27, 40 and 52-53. Additionally, hepatic aminopyrine demethylase activity was measured on wk 52 in 5 rats/sex/dose. All animals were subjected to a detailed gross and histopathological examination (tissues/organs examined histologically are given in Appendix 1). Samples of fat, liver, plasma and urine from selected animals were analysed for metabolites of the test chemical at weeks 26 and 76 and at study termination.

Results

The overall deviation from the target atmospheric concentrations was <2%. The 75 and 500 ppm airborne levels were equal to 450 and 3005 mg/m^3 respectively. Assuming that rats have a respiratory minute volume of 254 mL/kg bw (Mauderly *et al.*, 1982) and that the lung absorption rate for paradichlorobenzene is 33% in rats (EU, 2004), the respective daily systemic doses would have been 11 and 75 mg/kg bw/d.

No overt clinical signs were reported and there was no treatment-related effect on mortality, food or water intake or bodyweight. Although 'some changes' (unidentified) in clinical chemistry and haematology parameters attained statistical significance, there was 'no evidence of a dose-related change'. Supporting data were omitted. Hepatic aminopyrine demethylase activity did not increase. Urinary coproporphyrin and protein excretion was 'slightly' elevated at 500 ppm, which the study authors considered may have been related to functional changes in the liver or kidneys, which became enlarged although there was no histological evidence of effects in these organs. 'Small increases' in the weights of lungs and heart at 500 ppm were claimed to be unrelated to any histological change and discounted from being treatment-related. Apart from 'some suggestions' of increased liver weight (which can not be evaluated independently as data were not included), no changes from control values were discerned at 75 ppm. There was no carcinogenic effect of treatment.

The NOEC in this study was 75 ppm, based on liver and kidney enlargement and increased urinary coproporphyrin excretion at the high exposure concentration (500 ppm). Accordingly, the NOEL was *ca* 11 mg/kg bw/d. However, given the lack of reporting transparency and detail, and the relatively short duration of treatment (18 months as opposed to the 24 months now required by the US EPA and OECD for carcinogenicity studies) the study is not suitable for use in deriving a TDI for humans.

24-month inhalation study in rats (Aiso at al., 2005b)

In a study performed according to OECD Test Guideline 453 and GLP requirements, groups of 50 male and female F344/DuCrj rats (from Charles River Japan, 6 wk old, bw not stated, fed CRF-1 pellet diet [Oriental yeast Co, Tokyo Japan] and sterile water *ad libitum*) were exposed by inhalation to vapours of paradichlorobenzene (Wako Pure Chemical Industries Ltd, Tokyo Japan; purity ≥99.9%) at nominal concentrations of 0, 20, 75 or 300 ppm for 6 h/d, 5 d/wk for 104 wk. Control groups inhaled air only. Animals were caged individually within steel exposure chambers throughout the entire exposure period. No urinalysis, haematology or clinical chemistry investigations were performed.

All animals were necropsied, during which organs were weighed and examined macro- and microscopically. The organs examined were not identified but included the liver, kidney and transverse sections of the nasal passage.

Results

The mean achieved exposure concentrations were 19.8, 74.8, and 298 ppm, equal to 119, 450 and 1793 mg/m^3 respectively. Assuming that rats have a respiratory minute volume of 254 mL/kg bw (Mauderly *et al.*, 1982) and that the lung absorption rate for paradichlorobenzene is 33% in rats (EU, 2004), the respective daily systemic doses would have been 3.6, 14 and 54 mg/kg bw/d.

There were no treatment-related effects on bodyweight or food consumption. Survival of treated females was not compromised but the survival rate of 300 ppm males was only *ca* half as high as among controls (Table 5.4). The finding was claimed to be significant by Logrank analysis but a *p* value was not provided. Of the 32 male deaths at 300 ppm, 10 were attributed to leukaemia, 9 were caused by other neoplasms in various organs, 11 arose from chronic progressive nephropathy and a further 2 deaths had an unconfirmed cause. By comparison, in the male control group there were 3 leukaemia-related deaths, 8 deaths caused by other neoplastic disease in various organs and 6 deaths from chronic progressive nephropathy. Causes of death in females and the 20 and 75 ppm male groups were not stated.

At 300 ppm, absolute and relative liver weights were significantly increased in both sexes, as were absolute and relative kidney weights in males. However, no macroscopic lesions were noted in these or other organs. Microscopically, centrilobular hepatocellular hypertrophy was observed in a few animals at 300 ppm but was absent from controls and groups treated at lower doses. There was a dose-relatedly increased incidence of slight renal urothelial hyperplasia that was statistically significant in the 300 ppm male group, which also displayed papillary mineralisation in most animals (see Table 5.4). Notwithstanding this finding and the apparent excess mortality from chronic progressive nephropathy among 300 ppm males, at a histological level neither the incidence nor severity of the disease became enhanced in any of the treated groups. Furthermore, there was no evidence of treatment-related excess α_{2u}-globulin deposition in the kidney. Treatment-related cancer formation in the liver and kidney did not occur.

Paradichlorobenzene treatment caused histological abnormalities within the nasal tract at 75 and 300 ppm, to which males were relatively insensitive. This was seen as a marked increase in respiratory metaplasia of the nasal gland in 300 ppm females, together with an increased abundance of eosinophilic globules within the respiratory epithelium (Table 5.4). There was also a treatment-related increase in the density of eosinophilic globules within the olfactory epithelium in both sexes at 300 ppm and in 75 ppm females, although the total incidence of rats displaying globules in this location was unaffected. The globules were abundant in the ciliated and non-ciliated cells of the respiratory epithelium and in the supporting cells of the olfactory epithelium. Increased numbers of eosinophilic globules were associated with a marked reduction in the number of olfactory cells in the olfactory epithelium of 300 ppm females.

Table 5.4: Findings in rats inhaling paradichlorobenzene vapour for 24 months

Parameter	Sex	Atmospheric Concentration (ppm)			
		0	**20**	**75**	**300**
Mortality (n/ 50)	M	17	16	21	32
	F	12	16	12	14
Organ weights					
Liver, Absolute (g)	M	13	14	14	15**
	F	7.8	8.0	8.2	9.1**
Liver, Relative (% of bw)	M	3.3	3.6	3.6	4.0**
	F	2.6	2.7	2.8	3.2**
Kidney, Absolute (g)	M	3.0	3.2	3.2	3.6**
Kidney, Relative (% of bw)	M	0.78	0.80	0.85	0.97**
Liver histology (n/50)					
Hepatocellular hypertrophy;	M	0	0	0	5^
centrilobular	F	0	0	0	3
Kidney histology (n/50)					
Papillary mineralisation	M	0	1	0	41^^
Urothelial hyperplasia (pelvis)	M	7	8	13	32 ^^
Nasal cavity histology (n/50)					
Respiratory metaplasia:					
Nasal gland	F	5	4	4	33^^
Eosinophilic globules:					
Respiratory epithelium (slight):	F	11	10	14	38^^
Olfactory epithelium (slight):	M	32	20	19	19
	F	22	17	7	3
Olfactory epithelium (moderate):	M	1	1	1	7
	F	21	27	16	27
Olfactory epithelium (severe):	M	0	1	1	0
	F	6	2	23	20
Leukaemia (overall % incidence)	M	18	28	20	26

*P≤0.05; **P≤0.01 (Dunnett's Test); ^^p≤0.05; ^^p≤0.01 (Chi-square Test)

Despite the significant excess in leukaemia deaths among the 300 ppm male group, the study authors did not ascribe the disease to treatment because there was no dose-response relationship (see Table 5.4). Historical control data may have assisted in interpretation, but was not given. However, the study authors' interpretation is reasonable given that spontaneous development of leukaemia is relatively common in male F-344 rats at other laboratories, including facilities used by the US NTP.

Furthermore, the above gavage carcinogenicity study found no evidence of leukaemia formation at 150 mg/kg bw/d and did not demonstrate an unequivocal response even at 300 mg/kg bw/d (compared with the maximum achieved dose of 54 mg/kg bw/d in this inhalation study).

Based on histological abnormalities within the nasal passages at 75 ppm and above, the NOEC was 20 ppm, approximately equivalent to an oral dose of 3.6 mg/kg bw/d. If the findings within the nasal cavity are considered to be a portal-of-entry effect arising from localised irritation, a NOEC for systemic toxicity may be set at 75 ppm (14 mg/kg bw/d) based on increased liver and kidney weights, hepatocellular hypertrophy, renal papillary mineralisation and renal pelvic urothelial hyperplasia at 300 ppm (54 mg/kg bw/d).

12-month gavage study in rabbits (Pike, 1944)

In this study of ocular toxicity, paradichlorobenzene was administered orally (via stomach tube) to rabbits at 500 or 1000 mg/kg bw/d for 5 d/wk. The high dose caused marked intoxication, weakness, tremor, bodyweight loss and mortality in an unspecified proportion of the group. Rabbits tolerated the low dose for up to 12 months but displayed tremor and other [unstated] signs of intoxication. There were claimed to be no treatment-related effects on the eye but it is unclear what clinical examinations were performed, or when. A NOEL was not demonstrated.

12-month gavage study in rabbits (Hollingsworth et al., 1956)

Groups of 5 – 7 'white and coloured' rabbits received paradichlorobenzene (as a 25% solution in olive oil) via stomach tube at doses of 0, 500 or 1000 mg/kg bw/d, 5 d/wk. The high dose was administered up to 92 times over 219 days, while 263 low dose treatments were given over 367 days. Controls were treated with vehicle alone, for an unstated duration. 'Some' deaths occurred at 1000 mg/kg. Both doses caused weight loss, definite to marked tremors, weakness and 'slight changes' in the liver characterised by cloudy swelling with a few areas of focal caseous necrosis. No cataracts were produced. Haematological parameters in the 500 mg/kg group were 'within the normal range' but there is no evidence the relevant parameters were examined at the high dose. The study did not demonstrate a NOEL.

12-month oral capsule study in dogs (Naylor et al., 1996; assessed by EU, 2004 and US EPA, 2006)

In a one-year oral toxicity study (GLP) in beagle dogs, paradichlorobenzene was administered via capsule at doses of 0, 10, 50 or 150 mg/kg bw/d (5 dogs/sex/dose). Due to severe toxicity at 150 mg/kg, the initial dose of was adjusted to 100 mg/kg bw/d at the third week and 75 mg/kg bw/d at the sixth week. Blood and urine were collected pre-treatment, at 6 months and at termination for haematology, urinalysis and clinical biochemistry (parameters unknown). Organs examined histologically at necropsy are listed in Appendix I.

Results

One control dog died on day 83 due to jejunal displacement. At the highest dose (150 then 75 mg/kg) there was hypoactivity, emesis, dehydration, emaciation and decreased bodyweight gain during the first month of treatment. Two males and 1 female at 150 mg/kg died (the males on days 12 and 25, and the female on day 24) with congestion or haemorrhage in the intestine, lung or lymph node. One male and 1 female died from inflammatory lung lesions, associated in the female with pulmonary haemorrhages: the possibility that death was treatment related cannot be excluded. The cause of the death of the third animal was not clearly determined. As pulmonary inflammation in dogs and can be caused by nematode parasites, these were searched for but not detected.

At 6 months, mild anaemia was observed in both sexes at the HD, and platelet count was increased in MD and HD females. Basophil count was reduced at the high dose. At termination, platelet count was increased in HD females and MCV was elevated in MD males. Bone marrow erythroid hyperplasia (in 1 female) and excessive splenic haematopoiesis (in 2 females and 1 male) were also observed at the HD.

Statistically significant ($p<0.05$ or 0.01) dose dependent increases in absolute and relative liver weight occurred in HD (by 40–70%) and MD (by 25–55%) dogs of both sexes. A statistically significant, dose dependent increase in liver enzyme activity was reported. AP and ALT were elevated by 2–9-fold at the MD and HD, and AST and GGT were increased by up to 3-fold. Direct and total bilirubin, glucose and potassium were elevated, while creatinine, albumin and cholesterol were decreased in the HD females.

Histological findings in liver comprised hepatocellular hypertrophy in all males and females from the MD and HD groups (with hepatocellular pigment deposition in 2 males and 1 female at each dose); bile duct hyperplasia in 1 male and 1 female at the HD; and hepatic portal inflammation in 2 males and 1 female of the HD group. Renal discolouration and increased kidney weights occurred in HD and MD females and kidney duct epithelial vacuolisation was reported in 1 HD male and 4 females (1, 1 and 2 at the LD, MD and HD respectively). These were considered as being treatment-related only at the MD and HD, due to the accompanying renal enlargement and discolouration. Statistically and/or biologically significant increases in absolute and relative adrenal weight (by *ca* 25–58%) and thyroid weight (by *ca* 18–49%) occurred in MD and HD animals, but no histopathological lesions were found.

Based on the EU and US EPA reports, a NOEL can be set at 10 mg/kg bw/d. The LOEL in this study was 50 mg/kg bw/d, at which dose there was increased liver and kidney, adrenal and thyroid weights, haematological abnormalities, elevated serum enzyme activity, abnormal renal pathology and hepatocellular hypertrophy.

6 Genotoxicity Studies

The following section contains summaries of genotoxicity studies performed with paradichlorobenzene. This assessment covers published studies together with representative examples of the various types of studies evaluated in monographs published by the EU (2004), US EPA (2006) and WHO (1991).

Major genotoxicological end-points are included here, but for a complete account of the 50+ studies that have been reviewed in the scientific literature (including those of low significance, poor quality, and/or using un-validated protocols), readers are referred to the above reports.

Table 6.1: Mutagenicity of paradichlorobenzene in bacterial test systems

Test system	Test conditions	Results	Remarks	Reference
S. typhimurium strains TA98, TA100, TA1535, TA1537	Duplicate experiments ; Triplicate plates; DMSO vehicle; 1.0, 3.3, 10, 33 & 100 µg/plate (all strains, ± rat and hamster S9)	Negative	Assay lacked bacterial strain sensitive to point mutation at A-T sites. Test chemical manufactured by Aldrich, purity 97%. Sensitivity confirmed by positive controls.	Haworth *et al* (1983)
S. typhimurium strains TA98, TA100, TA1535, TA1537	Single experiment; Bacteria exposed to vapour at 94, 299 & 682 ppm. Duplicate experiments; DMSO vehicle; 4, 20, 100, 500 & 2500 µg/plate (all strains, ± rat S9)	Negative Negative	 Positive response (more than 2X negative control mutation frequency) in TA 1535 +S9 in 1 experiment but not the replicate. Sensitivity confirmed by positive controls.	Loeser and Litchfield (1983)
S. typhimurium strains TA98, TA100, TA1535, TA1537, TA1538	Triplicate experiments; Triplicate plates; DMSO vehicle; 51, 102, 204, 410, 819, 1638, 3277, 6553 & 13105 µg/plate (all strains, ± rat S9)	Negative	Test chemical manufactured by Tokyo Kasei Kogyo, purity 99%. Sensitivity confirmed by positive controls.	Shimizu *et al* (1983)
S. typhimurium strains TA98, TA100, TA1535, TA1537	Duplicate experiments ; Triplicate plates; DMSO vehicle; 5 concentrations over the range 1.0 – 100 µg/plate (all strains, ± rat and hamster S9)	Negative	Test chemical from Dover Chemical Co, >99% purity. Assay lacked bacterial strain sensitive to point mutation at A-T sites. Strains TA98, 1535 and 1537 showed no cytotoxicity at max concentration. Sensitivity not confirmed by positive controls.	US NTP (1987)
S. typhimurium strains TA98, TA100, TA1535, TA1537, TA1538	Highest concentrations were 2000 & 3000 µg/plate (± exogenous metabolic system)	Negative	Assessed by EU (2004). OECD guideline and GLP-compliant study. Test chemical from E Merck, purity unknown.	Winkler *et al* (1993)

Table 6.2: Mutagenicity of paradichlorobenzene in yeast or fungal test systems

Test system	Test conditions	Results	Remarks	Reference
Aspergillus nidulans meth3 locus reverse mutation	Single experiment; tested at 0 or 200 µg/mL without metabolic activation.	Positive	Assessed by EU (2004). Source, purity of test chemical unknown.	Prasad (1970)
Saccharomyces cerevisiae D7 strain, reverse mutation	Tested at 0, 74, 147 or 588 µg/mL (± mouse liver S9)	Negative -S9 Positive +S9	Assessed by EU (2004). Source, purity of test chemical unknown.	Paolini *et al* (1998)

Table 6.3: Mutagenicity of paradichlorobenzene in mammalian cells *in vitro*

Test system	Test conditions	Results	Remarks	Reference
L5178Y/TK$^{+/-}$ mouse lymphoma cells	Duplicate experiments; duplicate or triplicate plates; DMSO vehicle; 3 concentrations over the range 65-95 µg/mL (-rat S9) & 7 concentrations over the range 65-100 µg/mL (+rat S9); 4h exposure period; 48 h expression period	Negative	Test chemical from Dover Chemical Co, >99% purity. Cytotoxicity at ≥85 µg/mL (-S9) and at ≥65 µg/mL (+S9). Sensitivity confirmed by positive controls.	US NTP (1987)
Chinese hamster ovary cells	± exogenous metabolic activation system; otherwise not stated	Negative	Assessed by WHO (1991)	Bayer AG (1986a)
Chinese hamster ovary cells	Concentration range 70-350 µg/mL (±S9); 4-h exposure period; 7-d expression period	Negative	Assessed by EU (2004). Chemical from Bayer AG, purity unknown. Cytotoxicity at >240 µg/mL	Den Boer & Hoorn (1986a)

Table 6.4: Mutagenicity of paradichlorobenzene in mammals *in vivo*

Test system	Test conditions	Results	Remarks	Reference
♂CD-1 mice	Dominant lethal mutation assay. Animals exposed to vapour at 0, 75, 225 or 450 ppm for 6 h/d for 5 d. Each ♂ mated with 8 ♀, over 2 mo. Uteri examined on GD 13.	Negative	Doses were *ca* 120, 360 & 720 mg/kg bw/d. No treatment-related increase in dominant lethal mutations or effect on male fertility. Sensitivity confirmed by positive controls.	Loeser and Litchfield (1983)

Table 6.5: Induction of cellular transformation *in vitro*

Test system	Test conditions	Results	Remarks	Reference
Balb 3T3 cells, focus assay	Cells exposed for 72 h at 60-140 µg/mL then cultured for 4 wk.	Negative	Assessed by EU (2004). Chemical from Bayer AG, purity unknown.	Den Boer & Hoorn (1986b)
Balb 3T3 cells	Not stated	Negative	Assessed by WHO (1991)	Milone (1986b)

Table 6.6: Induction of unscheduled DNA synthesis *in vitro*

Test system	Test conditions	Results	Remarks	Reference
Hela cells	± exogenous metabolic activation system; otherwise not stated.	Negative	Assessed by WHO (1991)	Milone (1986a)
Human lymphocytes	Tested at up to 147 µg/mL ± S9; 4-h exposure	Negative	Assessed by WHO (1991) & EU (2004). Test chemical source unknown, purity 99%.	Perocco *et al* (1983)

Table 6.7: Induction of unscheduled DNA synthesis *in vivo*

Test system	Test conditions	Results	Remarks	Reference
B6C3F1 mouse & F344 rat liver & kidney	Doses were 0, 300, 600 or 1000 mg/kg bw PO. Cells harvested at 16-96 h.	Negative for UDS induction Positive for SPS	Assessed by EU (2004). Significant increase in S-phase DNA replication in ♂&♀ mouse liver & ♂rat kidney. Chemical from Standard Chlorine, purity 99.5%	Sherman *et al* (1998)
B6C3F1 mouse liver	Doses were 300, 600 or 1000 mg/kg bw PO. Hepatocytes harvested 16 & 48 h later.	Negative for UDS induction Positive for SPS	No increase in UDS. Small but statistically significant increase in S-phase DNA replication in ♂ only. Assessed by WHO (1991)	Steinmetz & Spanggord (1987)

Table 6.8: Induction of DNA strand breakage *in vitro* and *in vivo*

Test system	Test conditions	Results	Remarks	Reference
♀♂Hsd:Sprague Dawley rat kidney (Comet assay *in vivo*)	Rats dosed at 0, 1000 & 2000 mg/kg bw PO, kidneys harvested at 16 or 24 h	Weak positive in females Equivocal in males	Assessed by EU (2004). Strongest evidence of DNA damage at 16 h in females. Chemical from Bayer AG, purity unknown.	Brendler-Schwaab (2002)
Rat & human primary hepatocytes (alkaline elution assay *in vitro*)	Tested at 470 µg/mL without exogenous metabolic activation system; 20-h exposure.	Negative	Assessed by EU (2004). Test chemical from Aldrich Chimica, purity 99%.	Canonero *et al* (1997)
Rat & human kidney cells (Comet assay *in vitro*)	Tested at up to 823 µg/mL without exogenous metabolic activation system; 20-h exposure	Positive with dose-response in rat & human cells	Assessed by EU (2004). Test chemical from E Merck, purity 99%.	Robbiano *et al* (1999)
♂CD-1 mouse, various tissues (Comet assay *in vivo*)	Mice dosed at 2000 mg/kg bw IP, organs harvested at 0, 3 & 24 h	Positive in liver & spleen Negative in lung, kidney, bone marrow	Assessed by EU (2004). DNA damage present at 3 h only. Test chemical from Waco Pure Chemical Industries, purity unknown.	Sasaki *et al* (1997)

Table 6.9: Clastogenicity of paradichlorobenzene in mammalian cells *in vitro*

Test system	Test conditions	Results	Remarks	Reference
Rat & human primary hepatocytes	Rat cells exposed at 147 & 265 µg/mL No metabolic activation. Human cells exposed at 82-470 µg/mL	Weak positive for micronucleus induction Negative	Assessed by EU (2004). Test chemical from Aldrich Chimica, 99% purity. Cytotoxicity not estimated.	Canonero *et al* (1997)
Human lymphocytes	± exogenous metabolic activation system; otherwise not stated	Negative	Assessed by WHO (1991)	Milone (1986b)
Rat & human primary kidney cells	Cells exposed at up to 823 µg/mL without metabolic activation.	Positive for induction of micronuclei	Assessed by EU (2004). Test chemical from E Merck, purity 99%. Dose-related increase in micronucleated cell frequency over 265-823 µg/mL. Cytotoxicity not estimated.	Robbiano *et al* (1999)
Chinese hamster lung fibroblast cells	Not stated	Negative	Assessed by WHO (1991)	Sofuni *et al* (1985)
Chinese hamster ovary cells	Apparently single experiments; DMSO vehicle; Concentrations for induction of SCEs were 75, 100, 125 & 150 µg/mL (-rat S9) and 100, 125 & 150 µg/mL (+rat S9); 24-26 h exposure period –S9 or 2 h +S9; respective expression periods were 2-3 and 26 h. Concentrations for induction of aberrations were 50, 100 & 150 µg/mL (-rat S9) and 25, 50 & 100 µg/mL (+rat S9); 8-10 h exposure period (-S9) or 2 h + S9; respective expression periods were 2-3 h and 8-10 h.	Negative for induction of SCEs. Equivocal for induction of aberrations –S9, negative +S9.	Mitotic indices not reported for either end-point. Sensitivity to both end-points confirmed by positive controls. Test chemical from Dover Chemical Co, >99% purity. Positive result for aberrations –S9 based on only one observation at a single concentration (5% cells with aberrations @ 150 µg/mL *vs.* ≤2% @ ≤100 µg/mL).	US NTP (1987)

Table 6.10: Clastogenicity of paradichlorobenzene in mammals *in vivo*

Test system	Test conditions	Results	Remarks	Reference
Mice	Dose was 2500 mg/kg bw PO. Bone marrow harvested at 24, 48 & 72 h.	Negative	Assessed by WHO (1991)	Herbold (1986)
Mice	Doses were 355 & 710 mg/kg bw given divided as 2 IP injections separated by 24 h. Bone marrow harvested at 6 h.	Negative	Reduction in erythropoiesis occurred at both doses. Assessed by WHO (1991)	Herbold (1988)
NMRI mice, ♂	No. of micronucleated cells/1000 PCEs in femoral bone marrow counted in 5 mice/group. Doses were 0, 355, 710, 1065 & 1420 mg/kg bw given divided as 2 IP injections separated by 24 h.	Positive	Test chemical from Merck Co, 99% purity. 5 experiments/dose. Dose-related increase from 4.0 - 6.6 micronuclei/1000 PCE over 355 – 1420 mg/kg bw, vs. 1.8 micronuclei/1000 PCE in vehicle controls. At all doses $p<0.01$ Highest dose was 70% of the IP LD_{50}. NCE data not provided. Sensitivity confirmed by positive control.	Mohtashamipur *et al* (1987)
Mice from 1st NTP 13-wk study	No. of micronucleated cells/1000 RBC in peripheral blood counted in 1-10 mice/sex/group. Doses were 0, 600 (♂ only), 900 (♂ only), 1000 (♂ only), 1200 (♀ only), 1500 & 1800 mg/kg bw/d.	Negative	Test chemical from Dover Chemical Co, >99% purity. Non-standard protocol using subchronic gavage dosing. 7/10 ♂ and 9/10 ♀ deaths at HD but duration of exposure of decedents unknown. PCE and NCE data not provided. Sensitivity not confirmed by positive controls.	US NTP (1987)
Alderley Park rats, sex not stated	Single 2-h exposure at 299 or 682 ppm Exposure for 5 h/d for 5 d at 75 or 500 ppm Exposure for 5 h/d for 3 mo at 75 or 500 ppm Animals killed 22 h after final exposure for examination of bone marrow smear, 50 or 100 cells/rat.	Negative	Doses were *ca* 18 & 41 mg/kg bw for the 2-h exposure experiment, and 11 & 75 mg/kg bw/d in the repeat-dose experiments. PCE and NCE data not provided. Sensitivity confirmed by positive control.	Loeser and Litchfield (1983)

Kokel *et al.,* (2006): The anti-apoptotic activity of paradichlorobenzene was studied in the nematode *Caenorhabditis elegans*. The experimental model utilised *ced-3(n2438)* worms which carry a partial loss of function mutation in the *ced-3* gene coding for CED-3 caspases, the key enzymatic executioners of apoptosis. Worms were exposed to paradichlorobenzene vapour at an unspecified concentration for 48 hours during development from the embryo stage until the L4 larval stage, or continuously for 1 generation from the L4 larval stage to L4 F_1 progeny (duration of exposure unstated). In addition, embryos from exposed parental generation worms were removed from the test atmosphere 1 hour after being laid and exposed for 48 hours until development to L4 larvae. Inhibition of apoptosis was measured in L4s by counting the number of inappropriately surviving cells in the worms' anterior pharynx. (Normally, these 'survivors' would undergo apoptosis and be lost during embryonic development.) Further nematodes were cultured on plates overlaid with soybean oil in which paradichlorobenzene was dissolved at concentrations ranging from 25–250 mM.

Results

Control (air-exposed) *ced-3(n2438)* larvae had a mean of *ca* 1.5 supernumerary pharyngeal cells, as did unexposed larvae whose parents had been held in paradichlorobenzene vapour. By contrast, larvae from the 1-generation continuous exposure and 48-hour exposure groups had 3.3–3.8 extra cells. Therefore, the effects of paradichlorobenzene were not heritable, given that apoptotic inhibition occurred only in worms directly exposed during development from embryos to the L4 stage.

There was a linear, concentration-dependent increase in the number of supernumerary cells in *ced-3(n2438)* larvae cultured with oil containing the test chemical. This ranged from *ca* 1.7 to 6 extra cells in the vehicle control and 250 mM groups, respectively. The same effect was also observed in other apoptosis-deficient mutant strains of *C. elegans*, but not in wild type apoptosis-competent worms. A number of additional end-points were investigated. The test chemical (at 250 mM):

- reduced the number of apoptotic cell corpses seen in wild type embryos by up to 50%;
- caused a similar decrease in the number of persistent cell corpses present in engulfment-defective *ced-1(e1735)* mutant embryos;
- inhibited the death of male-specific cephalic companion neurons in 6% of wild type worms (*vs.* 0% inhibition in vehicle controls) and 66% of *ced-3(n2438)* mutants (*vs.* 60% inhibition in vehicle controls); and
- also in *ced-3(n2438)* larvae, increased the number of surviving ventral cord cells from an average of 2.5 in vehicle controls to *ca* 5.

The results suggested that paradichlorobenzene-mediated inhibition of apoptosis is dependent neither on the nature of the mutations nor on mutations in a specific cell-death gene. In addition to inhibiting apoptosis, paradichlorobenzene mediated some non-specific toxic effects including developmental delays, reduction in brood size and some lethality at the same concentrations as those affecting apoptosis. However, no further information was provided. The study authors suggested that the biochemical mechanisms controlling apoptosis in nematodes and mammals are sufficiently similar for there to be a common mechanistic basis for the effects seen in *C. elegans* and the carcinogenicity of paradichlorobenzene *in vivo*.

Lattanzi *et al.*, (1989): Thirteen Wistar rats and 47 BALB/c mice (adult males from Charles River, Como, Italy; weighing 250–300 and 22–28 g respectively), received IP injections of 127 µCi (2.95 µmol or 0.43 mg/kg bw) of ^{14}C-labelled paradichlorobenzene in ethanol. A further 3 rats and 12 mice served as untreated controls. Fasted animals were killed 22 or 72 h post-treatment and the concentration of radioactivity in liver, kidney, lung and stomach was measured by LSC. Chemical and radioactivity binding to DNA, RNA and protein within these tissues (individual tissues from each rat and pooled tissues from groups of 11-12 mice) were measured by LSC, UV spectrophotometry and colorimetry. In a further experiment, 4 rats and 12 mice were pre-treated for 2 days with phenobarbitone (100 mg/kg bw/d IP) before injection with ^{14}C-paradichlorobenzene and measurement of macromolecule binding *in vivo* as described above. Interaction between paradichlorobenzene and microsomal and cytosolic fractions of liver, kidney, lungs and stomach from phenobarbitone-pre-treated and non-pre-treated rats and mice was also studied in an *in vitro* test system which measured the extent of binding to calf thymus DNA.

Results

Radioactivity was present in rat and mouse tissues at 'low' levels 22 hours post-administration, but the concentrations in rat tissues were 2- to 10-fold higher than in mouse tissues (no further data provided).

Binding levels at 22 hours are shown in Table 6.11. The test chemical bound most extensively to RNA and least extensively to DNA. Indeed, paradichlorobenzene did not bind to DNA in rat tissues, whereas there was detectable binding to DNA in mouse tissues (especially lung). The covalent binding index value for mouse liver DNA was 14, but the study authors did not provide further binding index values for the test chemical on DNA, RNA or protein. Binding to RNA occurred in both species, being most extensive in mouse lung and liver but similar in the kidney of both species. There were no marked inter-species differences in the extent of binding to protein within the kidney, lung and stomach but there was *ca* 6-fold more binding to mouse liver than rat liver protein.

Table 6.11: *In vivo* binding^ (pmol/mg) of ^{14}C-paradichlorobenzene to DNA, RNA and protein in rat and mouse organs 22 hours post-administration

Tissue	Species	DNA	RNA	Protein
Liver	**Rat**	0	0.60	0.12
	Mouse	0.14 then 0 @ 72 h	1.83 then 2.42 @ 72 h	0.75 then 0.07 @ 72 h
Kidney	**Rat**	0	2.28	0.63
	Mouse	0.09	1.60	0.74
Lung	**Rat**	0	0.95	0.60
	Mouse	0.60	4.28	0.73
Stomach	**Rat**	0	0.08	0.54
	Mouse	0.08	ND	0.38

^Values shown are net increases above control levels. Rat data are mean of 9 individual values. Mouse data are mean of 3 pooled samples, each containing tissue from 11–12 animals. ND = No data

The study authors claimed that phenobarbitone pre-treatment did not affect *in vivo* binding in mouse liver or kidney, but provided no supporting data. Also omitted were binding data from 72 hours post-treatment except for mouse liver.

Here, DNA binding was undetectable (suggesting that DNA adducts had been repaired), RNA binding was increased by 30%, and protein binding had declined by an order of magnitude (see Table 6.11).

In vitro, hepatic microsomes from rats and mice were equally active in catalysing the binding of ^{14}C-paradichlorobenzene to calf thymus DNA (both *ca* 80 pmol/mg). With mouse liver microsomes, addition of SKF-525A inhibited binding to DNA by 88% while GSH enhanced the process by 3-fold. This is consistent with microsomal mixed function oxidases and GSH transferases having a key role in creation of adducts in mouse liver. There was a marked difference between the activities of lung microsomes: those from rats had only low activity (<1 pmol binding/mg DNA), whereas those from mice were moderately active (*ca* 32 pmol/mg). Renal microsomes from both species were inactive. Cytosols from mouse liver and mouse and rat kidney and lung mediated low levels of DNA binding (*ca* 1–5 pmol/mg) but rat liver cytosol was inactive. Mixtures of microsomal and cytosolic fractions from rat and mouse liver showed synergistic activity (*ca* 160 and 100 pmol/mg, respectively). Synergy was also observed with lung microsome/cytosol mixtures (rats *ca* 16 and mice 410 pmol/mg). Renal microsome/cytosol mixtures from both species were relatively inactive
(<2 pmol/mg).

Comment: It is unfortunate that the study authors did not include a mutagenic carcinogen in the experiment as a positive control, which would facilitate judgements as to the biological significance of the observed binding of paradichlorobenzene *in vivo*. The study provides little evidence that the observed carcinogenicity in mouse liver and rat kidney arises through genotoxic, rather than by epigenetic processes.

Paolini *et al.,* (1998) (assessed by EU, 2004) incubated [^{14}C]-paradichlorobenzene with calf thymus DNA and various subcellular fractions of liver from male CD-1 mice that had been treated with either phenobarbitone and β-naphthoflavone (PB/NF) or butylated hydroxytoluene. After centrifugation to remove particulate material, DNA was extracted and radioactivity associated with it was measured. [^{14}C]-paradichlorobenzene (or its metabolites) was associated with DNA, particularly after incubation with microsomes from PB/NF-treated mice (+ NADPH + GSH), or S9 fraction (+NADPH + GSH) from either PB/NF – or BHT-treated mice. No negative or positive control chemicals were included in this non-validated study.

Tian *et al.,* (2001a) (assessed by EU, 2004) The results obtained by Paolini *et al.,* (see above) were contradicted by this study, in which calf thymus DNA was mixed with paradichlorobenzene, an NADPH generating system and microsomes from male Fischer 344/NSIc rats, male BDF1 mice or humans. DNA was extracted and analysed by ^{32}P-post-labelling after enhancement of assay sensitivity with nuclease P1. No DNA adducts were detected.

7 Reproductive Toxicity Studies

Bornatowicz (1994) (assessed by the EU, 2004 and US EPA, 2006) conducted a two-generation study (according to OECD TG 416) on Sprague Dawley rats (24 rats/sex/dose) by gavage at 0, 30, 90, 270 mg/kg bw/d, 7 d/wk. F0 males were treated 77 days before mating, and F0 females were treated 14 days before mating and during mating, lactation and gestation until PND 21. Groups of 21 weanlings/sex/dose were treated for 84 days before mating, followed by exposure of both sexes for 30 days during mating and females during gestation (21 days) and lactation (21 days).

Study endpoints included clinical observations in adults and pups, body weight and food consumption in maternal animals (during gestation and lactation) and pups (from birth to day 21), reproductive indices (including duration between mating and successful copulation, number of pregnancies, gestation length, and litter size), numbers of live and dead pups, postnatal survival, postnatal developmental milestones (times to erect ears and eyelid separation), and neurobehavioral effects in pups at weaning (auricle reflex, orientation reaction, grasping, and draw-up reflexes). Necropsies were performed on adult males and females at the scheduled sacrifices, on apparently non-pregnant F0 and F1 females and spontaneously dead animals, and on pups that died during the first 4 days or were sacrificed on PND 4 (i.e., those not selected for continuation in the study). Liver, kidney, and spleen weights were measured in males and females of both generations; it was not indicated if additional organs were weighed. Histopathologic examinations were limited to selected tissues (liver, kidneys, spleen, vagina, cervix, uterus, ovaries, mammary gland, testes, epididymides, penis, prostate, seminal vesicles, and spermatic cord) from adult F0 and F1 animals that had no living young, died prematurely, or were sacrificed as moribund, as well as gross lesions in all animals.

Results

No reproductive or other exposure-related changes were found at 30 mg/kg bw/day in adults or pups. Effects occurring at ≥90 mg/kg included statistically significantly reduced (method of analysis and p values not reported) average birth weight in F1 pups (4.4, 5.7, and 23% lower than controls at 30, 90, and 270 mg/kg, respectively). Significant reductions in body weight were also observed at 270 mg/kg in F1 pups on PND 7 to 21 and in F2 pups at birth and on PND 4 to 21. The total number of deaths from birth to PND 4 was significantly increased in F1 pups at 270 mg/kg (3/214, 6/310, 7/273, and 62/231 at 0, 30, 90, and 270 mg/kg, respectively), and F2 pups at ≥90 mg/kg (3/294, 4/296, 17/330, and 31/253 at 0, 30, 90, and 270 mg/kg, respectively). None of the data in this study were reported on a per litter basis or analysed for dose-related trends. Other significant effects on offspring survival indices were observed at 270 mg/kg, including reduced total number of live F1 and F2 pups at birth, increased total dead F1 and F2 pups at birth, and increased total dead F1 and F2 pups during PND 5 - 21.

Additional exposure-related effects included delayed eye opening (first day of appearance or day shown in all pups) in F1 and F2 pups at 270 mg/kg, delayed ear erection (day shown in all pups) in F2 pups at 270 mg/kg, and reduced percentage of pups per litter with a positive reaction in the draw-up test in the F1 pups at 270 mg/kg and in F2 pups at ≥90 mg/kg (3.3, 7.4, and 22% less than controls at 30, 90, and 270 mg/kg, respectively). Clinical manifestations were evident in pups of both generations at ≥90 mg/kg, including dry and scaly skin until *ca* PND 7 (0, 0, *ca* 70 and 100% of the litters at 0, 30, 90 and 270 mg/kg, respectively). Tail constriction appeared between PND 4 and 21 in all or nearly all litters (percentages not reported) and, in isolated cases, led to loss of parts of the tail. (Annular constriction of the tail results from a lack of hydration in the skin). Additionally, the number of F1 pups described as cyanotic after birth was increased (not quantified) at 270 mg/kg.

Effects observed in adult animals were generally not quantified, but included reduced average body weight in F1 males and females at 270 mg/kg (*ca* 20 g [males] or 10 g [females] lower than control groups at all time points during gestation and lactation [no other data reported]), increased relative liver weight in F1 males at ≥90 mg/kg, and increased absolute and/or relative kidney weights and reduced spleen weights in F1 males at 270 mg/kg.

There were no effects on organ weights in females of either generation. The only histopathological finding attributed to exposure was unspecified kidney damage in both generations (effect levels, possible male specificity, and other information not reported).

The EU reviewer concluded there was a NOAEL for fertility at 270 mg/kg bw/day; found the NOAEL for the F0 and F1 parents to be 90 mg/kg bw/day, and set a NOAEL for developmental effects at 30 mg/kg bw/day. The latter NOAEL was based on increased F1/F2 pup mortality, reduced mean birthweight in the F0/F1 generation and retarded physical and behavioural indices from 90 mg/kg bw/day. The US EPA reviewer agreed that there were no effects on mating and fertility indices in any group, and identified a NOAEL and LOAEL of 30 and 90 mg/kg bw/d, respectively, for developmental toxicity based on increased mortality and other effects in F1 and F2 pups during the pre-weaning period.

From the available information, the study appears to have had shortfalls in histological examination and reporting, which will have reduced its ability to detect evidence of renal injury or other organ toxicity in adults. However, in respect of mating and fertility indices alone, it is possible to assign a NOEL of 270 mg/kg bw/d for effects on the parental rats. There was a NOEL of 30 mg/kg bw/d for effects on pups, based on fetotoxicity, increased post-natal mortality, retarded neurobehavioural development and dermal lesions at 90 mg/kg bw/d.

Makita (2004) performed a 1-generation reproductive toxicity study in which paradichlorobenzene and p,p'-DDE were administered separately and in combination to pregnant rats. This evaluation will examine findings in the paradichlorobenzene-treated group.

Pregnant Wistar rats (6/group, 230 – 240 g bodyweight; from Kyudo Breeding Co, Tosu, Japan) were administered paradichlorobenzene (99.9% purity; manufactured by Tokyo Kasei Co, Tokyo Japan) at a concentration of 25 ppm in the diet, for 42 days commencing from GD 1 and ending on PND 21. A control group received plain diet (CE-2 rat chow; Clea Japan Inc, Tokyo Japan). Bodyweight and food consumption of dams were measured during gestation. Pups were examined for gross malformations on PND 1, and litters were culled to 4 pups/sex on PND 2. Ano-genital distance was recorded on PND 4 and 7. Pups were kept with their dams until weaning on PND 21, after which they received control diet and were weighed and assessed for developmental landmarks. Pups were sacrificed on PND 37 and necropsied. Organs including liver, kidneys, spleen, ovaries, uterus and thymus were weighed and examined histologically. Serum LH and FSH levels were assayed by radioimmunoassay.

Results

The achieved daily dose of paradichlorobenzene was 2.0 mg/kg bw/d, based on measurement of maternal food intake and test chemical concentration in the food. There were no treatment-related effects on dams, or on reproductive indices including litter size and pup sex ratio. Pup viability was not compromised by the test chemical, and no treatment-related effects occurred on pup birthweight, food consumption or growth and maturation. Pup LH and FSH concentrations were unaffected. In pups from treated dams, the only remarkable finding at necropsy was a slightly but significantly ($p<0.05$) increased mean absolute thymus weight (*ca* 0.65 vs. 0.55 g in controls). The biological significance of this is questionable, given that there were no accompanying histological abnormalities. Other organs from pups were also microscopically normal.

The NOEL for effects on foetuses and pups was 25 ppm (equal to 2.0 mg/kg bw/d), the sole dose administered. There was no evidence of maternotoxicity but the limited range of maternal observations precludes assigning a NOEL to dams.

Tyl and Neeper-Bradley (1989) (Assessed by EU, 2004 and US EPA, 2006) performed a two-generation study (GLP) on Sprague Dawley rats (28 rats/sex/dose) by inhalation exposure (vapour) at 0, 66, 211, or 538 ppm (6 h/d, 7 d/wk) over the 10 weeks before mating, during mating, gestation and lactation.

The 3 respective exposure concentrations were equal to 397, 1268 and 3233 mg/m^3. Assuming that rats have a respiratory minute volume of 254 mL/kg bw (Mauderly *et al.*, 1982) and that the lung absorption rate for paradichlorobenzene is 33% in rats (EU, 2004), the respective daily systemic doses would have been *ca* 12, 38 and 97 mg/kg bw/d.

Additional groups of 10 females were similarly exposed for 10 weeks in a satellite study. The animals in the main study were paired within groups for a 3-week mating period to produce the F1 generation. Main study males that did not mate successfully during the first 10 days of the mating period were paired with satellite females for 10 days. Main study females that did not mate successfully during the first 10 days of the mating period were paired with proven males for the remaining 11 days. Exposure of the main study F0 females was continued throughout mating until GD 19 days, discontinued from GD 20 through PND 4, and then resumed until sacrifice at weaning on PND 28. Exposure of the satellite F0 females was continued through mating until sacrifice on GD 15. Exposure of F0 males continued until sacrifice at the end of the study and satellite mating periods. Groups of 28 F1 weanlings/sex and satellite groups of 10 F1 female weanlings were exposed for 11 weeks and mated as described above to produce the F2 generation. Additionally, 20 F1 weanlings/sex from the control and high exposure groups served as recovery animals that were observed without exposure for 5 weeks prior to sacrifice. Complete necropsies were performed on all F0 and F1 parental animals, F1 recovery animals, F1 weanlings not used in the rest of the study and F2 weanlings. Histology was evaluated in the F0 and F1 parental animals on the liver and kidneys in all groups and on pituitary, vagina, uterus, ovaries, testes, epididymides, seminal vesicles, prostate, and tissues with gross lesions in the control and high exposure groups. The kidney evaluation included examination for the presence of α_{2u}-globulin droplets. Additional endpoints evaluated in the parental generations included clinical observations, mortality, body weight, and food consumption. Mating and fertility indices were determined for F0 and F1 males and females, and gestational, live birth, postnatal survival (PND 4, 7, 14, 21 and 28), and lactation indices were determined for the F1 and F2 litters.

Results

In the F0 and F1 adults at 538 ppm, there was reduced food consumption, increased incidences of clinical signs (tremors, scruffy appearance, urine stains, salivation, and nasal and ocular discharges), reduced gestational and lactational bodyweight gain; and post-natal toxicity evidenced by an increased number of stillborn pups, reduced pup bodyweights and reduced post-natal survival in F1 and/or F2 litters. No developmental effects were reported**.**

Pathological examination revealed hyaline droplet nephropathy in F0 and F1 adult males at ≥66 ppm. Manifestations of this male rat-specific renal syndrome included α_{2u}-globulin accumulation and increased kidney weights at ≥66 ppm and other characteristic histologic changes (e.g., tubular cell hyperplasia) at 538 ppm. Bodyweights and weight gain were significantly reduced in F0 and F1 adult males and F1 adult females during the pre-mating exposure period at 538 ppm. Relative liver weights were significantly ($p<0.05$ or $p<0.01$) increased in F0 adult males at ≥66 ppm, F0 adult females and F1 adult males at ≥211 ppm, and F1 adult females at 538 ppm.

Absolute liver weights were significantly increased in F0 adult males at ≥211 ppm, and in F0 adult females and F1 adults at 538 ppm. Liver weight changes were more pronounced in males than in females. Mean relative liver weights in the 66, 211, and 538 ppm adult male groups of the F0 generation (sacrificed at week 15) were 4.8, 14 and 52% higher than controls. Hepatocellular hypertrophy was observed in the livers of F0 and F1 males and females at 538 ppm but there were no accompanying degenerative lesions. No hepatic histologic changes were induced at the lower exposure concentrations.

The EU reviewer set a NOAEL for female rats (parents in F0 and F1 generations) of 211 ppm, established a LOAEL for the parental males at 66 ppm (due to hyaline droplet nephropathy) and set a NOAEL of 211 ppm in the offspring.

For the purposes of this assessment, a NOEL of 211 ppm (approximately equivalent to an oral dose of 38 mg/kg bw/d) is set for effects on offspring and in parental female rats. There was no NOEL in parental males due to male specific nephropathy at and above the lowest exposure level of 66 ppm.

8 Developmental Toxicity Studies

Giavini *et al.,* (1986) gavaged pregnant CD rats (20/group; from Charles River, Calco, Italy; bodyweight *ca* 190 g) with paradichlorobenzene (manufactured by Fluka, Switzerland; 99% purity) at 0, 250, 500, 750 or 1000 mg/kg bw/d (in corn oil at 2.5 mL/kg bw) on GD 6-15 inclusive. Dams were observed and weighed throughout gestation then sacrificed on GD 21, undergoing necropsy that included maternal reproductive parameters and liver weight, and foetal survival, growth and skeletal and visceral morphology.

Results

One dam from each of the 500 and 1000 mg/kg groups died due to 'technical error' but there was no chemical-related maternal mortality. During the treatment period, maternal bodyweight gain and food consumption were inhibited dose-relatedly, with the difference *vs.* controls attaining statistical significance at and above 500 and 250 mg/kg bw/d, respectively (see Table 8.1). There were no treatment-related effects on maternal liver weight or reproductive indices including post-implantation foetal losses.

Foetal survival was not compromised by treatment, but at 1000 mg/kg foetal bodyweight was significantly lower than the control value. paradichlorobenzene did not cause visceral anomalies or malformations. Although skeletal malformations were not seen, there was a treatment-related increase in the number of 750 and 1000 mg/kg group foetuses with skeletal anomalies due to formation of supernumerary ribs and/or hemi-sternebrae.

There was no maternal NOEL due to evidence of maternotoxicity (decreased food consumption) at and above the lowest dose of 250 mg/kg bw/d. The NOEL for effects on the foetus was 250 mg/kg bw/d, based on increased incidences of skeletal anomalies at 500 mg/kg bw/d and higher.

Table 8.1: Maternal and foetal observations in rats administered paradichlorobenzene

Parameter	Dose (mg/kg bw/d)				
	0	**250**	**500**	**750**	**1000**
Maternal findings					
Dams mated (n/20)	16	16	17	16	13
Dams pregnant at necropsy (n/20)	16	12	14	16	11
Bodyweight gain, GD 6-15 (g)	47	39	38*	37*	21**
Food consumption, GD 6-15 (g/rat/d)	27	24*	24*	23*	22*
Foetal findings					
No. live foetuses	236	176	182	248	168
Foetal bodyweight (g)	3.7	3.8	3.7	3.6	3.4**
Skeletal anomalies (no. and % incidence)	41 36%	29 34%	37 42%	61 50%*	42 51%*
Hemisternebrae (no. foetuses)	4	0	3	13	9
Supernumerary ribs (no. foetuses)	7	8	15*	35*	26*

*p<0.05 **p<0.01

Loeser and Litchfield (1983) reported a study in which groups of al least 20 pregnant rats were exposed to paradichlorobenzene vapour for 6 h/d from GD 6 – 15 inclusive at atmospheric concentrations of 0, 75, 200 or 500 ppm. Dams were weighed and observed throughout gestation and then caesarean sectioned on GD 21, undergoing necropsy that included maternal reproductive parameters and foetal survival, growth and skeletal and visceral morphology (each in half the foetuses).

Results

Maternal bodyweight gain was 'normal' and no clinical or pathological signs of toxicity were reported, with the possible exception of premature littering, which occurred in 1, 1 and 5 dams at 75, 200 and 500 ppm, respectively. According to the study authors, there were no adverse effects on numbers of implantations, resorptions, viable foetuses, runts or corpora lutea, mean foetal bodyweight, litter weight or sex ratios. There was also claimed to be no evidence of retarded foetal ossification, increased incidences of minor skeletal or visceral variations or malformations.

The study authors concluded that the NOEC for maternal toxicity and effects on foetuses was 500 ppm (3005 mg/m^3), approximately equivalent to an oral dose of 75 mg/kg bw/d assuming rats have a respiratory minute volume of 254 mL/kg bw (Mauderly *et al.*, 1982) and that the lung absorption rate for paradichlorobenzene is 33% in rats (EU, 2004).

Hayes *et al.*, (1985) exposed groups of 29 – 30 inseminated NZW rabbits (3.5 – 4.5 kg bw; from Langshaw's Rabbitry, Augusta, MICH USA) to paradichlorobenzene vapour at 100, 300 or 800 ppm (600, 1800 or 4800 mg/m^3) in separate chambers for 6 h/d from GD 6-18 inclusive. Control rabbits were exposed as described to filtered room air in identical chambers.

Dams were observed and weighed throughout gestation then sacrificed on GD 29, undergoing necropsy that included maternal reproductive parameters and liver and kidney weight, and foetal survival, growth and skeletal (in all foetuses) and visceral (in half the foetuses) morphology.

Results

The mean daily time-weighted average airborne concentration of paradichlorobenzene vapour in inhalation chambers was within 3% of target values. One 300 ppm rabbit died on GD 10. Necropsy revealed multiple large intrathoracic abscesses. The death was not considered to be treatment-related. There were no other remarkable changes in behaviour or appearance of dams, but dose-related maternal bodyweight loss occurred at 300 and 800 ppm during the first 3 days of dosing (Table 8.2). During the 10 days after cessation of treatment, there were compensatory increases in bodyweight gain in these same groups, so that overall gain from GD 6–28 was unaffected at 300 ppm but depressed by *ca* 12% at 800 ppm. While statistical significance was attained at 800 ppm only, the effect is considered biologically significant at 300 ppm also. Neither absolute nor relative maternal liver and kidney weights were affected.

Notwithstanding evidence of maternotoxicity at 300 and 800 ppm, there were no effects on embryo-foetal survival or bodyweight. The most notable finding was a statistically significant increase in the incidence of foetuses having abnormal development of the subclavian artery. Noting that the historical control incidence of retro-oesophageal right subclavian artery was 2% *of litters* (incidence/no. foetuses was not provided), and that the feature was a minor variation rather than a malformation, the study authors concluded that a teratogenic response had not occurred. The reviewing toxicologist considers that the finding is likely to be treatment-related, given that arterial abnormalities occurred in at least 5/28 litters whose dams were exposed at 800 ppm (an incidence of 17%), compared with a single control litter. The incidence of foetuses with skeletal anomalies was slightly higher at 800 ppm than among the remaining groups. As there was no increase in the number of affected litters, this is considered as being of equivocal biological significance by the reviewing toxicologist.

The NOEC for maternotoxicity was 100 ppm, due to bodyweight loss in 300 and 800 ppm dams at the onset of treatment. The foetal NOEC was 300 ppm based on an increased incidence of circulatory system variations at 800 ppm.

Approximate achieved doses of dams may be estimated from Calabrese (1991), who cites a respiratory minute volume of 800 cm^3 for a 2069 g rabbit (i.e. 387 cm^3 kg/bw). Assuming 100% absorption of paradichlorobenzene across rabbit lung (in the absence of any relevant experimental data) then during each 360 min exposure period the intake of test chemical would have been 84, 251 or 669 mg/kg bw at the 100, 300 and 800 ppm exposure levels, respectively. The NOEL for maternotoxicity would therefore have been 84 mg/kg bw and the foetal NOEL was 251 mg/kg bw.

Table 8.2: Maternal and foetal observations in rabbits exposed to paradichlorobenzene

Parameter	Atmospheric concentration (ppm)			
	0	**100**	**300**	**800**
Maternal findings				
No. of dams mated	30	30	29	30
No. of dams pregnant at necropsy	29	25	26	28
Maternal bw gain GD 6-8 (g)	8.0	2.0	-32	-82*
Maternal bw gain GD 19-28 (g)	63	97	126	189*
Maternal bw gain GD 6-28 (g)	248	286	259	217
Foetal findings				
No. foetuses examined skeletally	210	176	175	218
No. foetuses examined viscerally	115	94	93	119
No. foetuses (litters) with:				
Acephaly^	0	0	0	1 (1)
Omphalocele^	0	0	0	2 (2)
Forelimb flexure^	0	2 (1)	1 (1)	4 (2)
Right subclavian artery retro-oesophageal	1 (1)	0	1 (1)	6 (5)*
Right subclav art originating from pulmonary trunk	0	0	0	1 (1)
Fused vertebrae	1 (1)	1 (1)	0	2 (1)
Fused ribs	0	1 (1)	1 (1)	3 (1)
Shortened leg bones	0	0	0	2 (1)

*p<0.05; ^A single 800 ppm foetus had acephaly, omphalocele (umbilical hernia) and forelimb flexure

9 Mechanistic Studies

Aryoshi *et al*., (1975) administered paradichlorobenzene to groups of 6 female Wistar rats (100–120 g bodyweight, receiving Funahashi Nojyo F-II diet) orally (in 2% tragacanth solution at a dose volume of 5 mL/kg bw) for 3 days at 250 mg/kg bw/d. Controls received vehicle only. Animals were decapitated 24 hours after the final dose and the livers removed, the concentrations of glycogen, phospholipids and triglycerides were measured, and the activities of aminopyrine demethylase and alanine hydroxylase were assayed in microsomal preparations. δ-ALA synthetase activity was measured in liver homogenates and the concentration of δ-ALA was quantified in the protein-free supernatant.

Results

The test chemical had relatively few effects on the parameters examined, increasing the proportion of 16:0 phospholipid fatty acids to 20.1% of total present (*vs.* 17.2 % in controls; $p<0.05$) but causing no significant change in the total concentration of triglycerides, phospholipids or microsomal protein. Compared with control values, alanine hydroxylase and aminopyrine demethylase activities per 100 g bw were increased by 30% and 50%, respectively. These displacements from controls were not statistically significant, but may have been biologically significant given that the comparatively low group sizes would have limited the power of the test used. No treatment-related effect on δ-ALA synthetase activity occurred.

Carlson and Tardiff (1976) [assessed by WHO (1991)] reported that following oral administration to rats at doses of up to 40 mg/kg bw/d for 14 days paradichlorobenzene induced cytochrome-c-reductase, cytochrome P-450, *o*-ethyl *o*-*p*-nitrophenyl phenylphosphonothioate detoxification, glucuronyl transferase, benzopyrene hydroxylase and azoreductase.

Charbonneau *et al.*, (1989) treated Fischer-344 rats of both sexes (200 – 250 g bodyweight; from Charles River laboratories, Raleigh, NC USA) by gavage with 1,4-[^{14}C]dichlorobenzene (>98% purity, 11 mCi/mmol, from Sigma Chemical Co, St Louis, MO USA) at 300 or 500 mg/kg bw (in corn oil at 5 mL/kg bw). After 24 hours, animals were exsanguinated under anaesthesia and radioactivity levels were measured in the adipose tissue, liver and kidney. Kidney tissue was also examined histologically for the presence of hyaline droplets.

Results

Tissue radioactivity levels in adipose tissue (see Table 9.1) were 2 orders of magnitude higher than those in plasma, liver and kidney. Half the radioactivity in kidney was localised within the cell cytosol, within which paradichlorobenzene and, to a minor extent, its metabolite 2,5-dichlorophenol were bound reversibly to renal α_{2u}-globulin. Little or no radiolabel bound covalently with proteins in the plasma or liver.

Table 9.1: Distribution of radiolabel from 1,4-[^{14}C-dichlorobenzene] in plasma and tissues in rats (N = 3/group) 24 hours after a 500 mg/kg bw gavage dose

Plasma nmol eq/mL	Liver nmol eq/g	Fat nmol eq/g	Kidney nmol eq/g	Kidney cytosol nmol eq/g kidney	% of kidney paradichlorobenzene eq in kidney cytosol
201	458	13 617	535	266	50

After a single 500 mg/kg bw dose, paradichlorobenzene increased hyaline protein droplet formation in male (but not female) kidney. Mean histology scores for protein droplets were 1.2, 3.2 and <1.0 in male controls, treated males and treated females, respectively. Renal α_{2u}-globulin concentration in treated males increased to 2.4-fold above control values. By comparison, α_{2u}-globulin could not be detected in kidney from female rats.

When 7 consecutive daily 120 or 300 mg/kg bw PO doses of paradichlorobenzene were administered to male rats, there were dose-related increases in protein droplet density and renal cell proliferation, measured by [^{3}H]thymidine incorporation into kidney DNA (see Table 9.2). The study authors noted the similarity between the biochemical alterations and nephrotoxicity caused by paradichlorobenzene and those induced by 2,4,4-trimethy-2-pentanol, a component of unleaded petrol causing renal nephropathy and cancer in male rats. They hypothesised that the chemical-α_{2u}-globulin complex is resistant to catabolism by lysosomal proteases, and accumulation of the complex (seen as hyaline droplets) leads to cell death and proliferative repair activity.

Table 9.2: α_{2u}-globulin protein droplet formation and [^{3}H]thymidine incorporation into male rat DNA

Treatment	Mean score for droplets 24 h after final dose	[^{3}H]TdR incorporation (dpm/µg DNA)
Corn oil	1.0	94.3
1,4-DCB 120 mg/kg bw/d	2.2	139
1,4-DCB 300 mg/kg bw/d	3.5	159

(Gustafson *et al*, 1998) (Assessed by US EPA, 2006) evaluated the potential for paradichlorobenzene to promote liver tumours in rats in a subchronic initiation/promotion bioassay. Male F344 rats were given a single IP injection of 0.9% saline (12 animals) or 200 mg/kg bw of diethylnitrosamine (DEN) in saline (18 animals), followed by oral administration of paradichlorobenzene beginning 2 weeks later. Rats promoted with paradichlorobenzene were treated with doses of 0.1 or 0.4 mmol/kg bw/day (15 or 59 mg/kg bw/day) in corn oil by gavage for 6 weeks. Control rats were similarly treated with corn oil alone or DEN in corn oil. All animals were partially hepatectomised 1 week after the start of paradichlorobenzene exposure. The study was terminated at the end of week 8, and immunohistochemical analysis was performed to identify pre-neoplastic glutathione-S-transferase expressing foci in the liver. No paradichlorobenzene related increased incidence of hepatic foci was found, suggesting that the compound was not a liver tumour promoter in rats.

Lake *et al* (1997) investigated the mechanisms underlying the observed species and tissue differences in paradichlorobenzene-induced tumour formation. Male Fischer 344 rats and B6C3F1 mice (9 weeks old; from Harlan Olac, Bicester, Oxon UK) were gavaged with paradichlorobenzene (>99% purity; from Sigma-Aldrich Chemical Co, Poole, Dorset UK) for 5 d/wk for 1, 4 or 13 weeks. Dose levels were 0, 25, 75, 150 and 300 mg/kg bw/d in rats and 0, 300 or 600 mg/kg bw/d for mice. Controls received vehicle (corn oil) alone.

Results

The test chemical caused significant ($p<0.05–0.001$) dose-related increases in relative liver weight at $\geq$75 mg/kg in rats and $\geq$300 mg/kg in mice. This was associated with mild (in rats) and marked (in mice) centrilobular hepatocytic hypertrophy at the highest respective doses given to each species. Liver morphology at lower doses was not examined.

In both mice and rats throughout the treatment period paradichlorobenzene induced large dose-related increases in hepatic microsomal CYP 450 content (CYP2B and to a lesser extent CYP2A isoenzymes) and 7-pentoxyresorufin *o*-depentylase activity. Displacements from control values of some or all of these parameters reached statistical significance ($p<0.01$ or 0.001) at and above the lowest administered doses. On week 1, liver microsomal protein content and 7-ethoxyresorufin *o*-deethylase and erythromycin *n*-demethylase activity were elevated in 150 and 300 mg/kg rats and 600 mg/kg mice. (These enzymes were not measured subsequently.)

Replicative DNA synthesis was measured from intracellular incorporation of 5-bromo-2'-deoxyuridine (BrdU) delivered via osmotic pumps implanted during weeks 0--1, 3-4 and 12-13. In rat hepatocytes, labelling index values were increased to 255% of control in animals given paradichlorobenzene at 300 mg/kg bw/d for 1 week ($p<0.01$). The phenomenon was transient and limited to the high dose, however. No significant increases were observed in animals treated at $\leq$150 mg/kg during week 1 or in any dose group after 4 or 13 weeks' treatment.

In mouse hepatocytes, labelling increased to 475 and 1175% of control ($p<0.001$) and to 420 and 395% of control values ($p<0.01$ and <0.05) in animals treated at 300 and 600 mg/kg for 1 and 4 weeks, respectively. Labelling indices remained similar at 13 weeks, but a 3-fold increase in control labelling activity made between-group differences insignificant.

Significant ($p<0.05$-0.001) increases in relative kidney weight were observed in rats given ≥150 mg/kg for 4 and 13 weeks, whereas there was no such effect in mice even at the highest dose. At week 4, mouse kidney P_1/P_2 proximal tubule cell labelling increased transiently to 205 and 170% of control at 300 and 600 mg/kg, respectively ($p<0.001$). Otherwise, relatively little cellular proliferation was observed in mouse kidney, but the test chemical caused dose-related increases in rat renal P_1/P_2 proximal tubule cell labelling at all time points. These tended to grow with increasing time, against a progressive decline among controls. At week 4, labelling indices were 250, 400 and 475% of control value at 75, 150 and 300 mg/kg. By week 13, P_1/P_2 labelling indices in the 150 and 300 mg/kg groups attained 440 and 775% of control, while P_3 cell labelling was enhanced by 485% in the 300 mg/kg group. These displacements from control were highly statistically significant ($p<0.01$ or 0.001).

This study demonstrates that paradichlorobenzene stimulates cell replication in both rat and (especially) mouse liver, but only in rat kidney. The study authors suggested that promotional effects of enzyme induction may be important in the formation of liver tumours in mice. The observed inter-species differences in liver tumour formation may reflect the greater sensitivity of mice to tumour promotion by CYP2B enzyme induction. In respect of the kidney, the study provided further evidence that while paradichlorobenzene-induced α_{2u}-globulin nephropathy is associated with a sustained stimulation of cell replication in male rat proximal tubule cells, this effect is not observed in male mice.

Reid (1973) and **Reid and Krishna (1973)** compared the covalent binding properties of paradichlorobenzene and/or its metabolites in the rat kidney and liver. Male Sprague-Dawley rats (160-200 g bodyweight) received a single IP injection of ^{14}C-paradichlorobenzene (10-30 µCi of radioactivity) at 0.5 mmol/kg bw (74 mg/kg bw). Animals were then kept in metabolism cages for collection of urine for 6 or 24 hours post-injection before being killed by cervical fracture. Urinary excretion of radioactivity was quantified by LSC. Liver and kidney tissues were examined by light microscopy and autoradiography. Unbound radioactivity in tissue homogenates was removed by solvent extraction, and the quantity of any remaining covalently protein-bound radioactivity was measured by LSC. Additional experiments were run on hepatic tissue binding in animals that had been administered the CYP 450-inducing drug phenobarbitone (80 mg/kg bw/d IP) 72, 48 and 24 hours before treatment with the test chemical. Some phenobarbitone-pre-treated rats also received the CYP 450 inhibitor SKF-525A (75 mg/kg bw IP) 60 minutes before treatment.

Results

The 0.5 mmol/kg bw dose of paradichlorobenzene did not cause any histologically-detectable injury to either liver or kidney. Peak total radioactivity levels in liver were observed at 6 hours post-treatment, amounting to 179 nmol equivalents/g tissue.

Phenobarbitone induction increased the concentration by 64%. As shown in Table 9.3, the concentration of radioactivity bound covalently to liver protein at 6 hours was significantly lower, at 21 nmol/g or *ca* 10% of the total present in liver tissue. The corresponding covalent binding level in kidney was less than half of that in liver. (By comparison, following an equivalent dose of the more hepatotoxic and nephrotoxic isomer ortho-dichlorobenzene, the respective covalent binding levels in liver and kidney were 234 and 62 nmol/g protein).

CYP 450 induction with phenobarbitone *decreased* the extent of binding to liver protein by half, while a combination of phenobarbitone induction and SKF-525-A co-treatment reduced the value by a further 50%. Effects of enzyme induction or inhibition were not investigated in kidney. Over a 24-hour period, both un-induced and phenobarbitone pre-treated rats excreted 24-29% radioactivity equivalents of the administered dose via the urine. SKF-525A did not influence either the total radioactivity level in liver or urinary excretion of paradichlorobenzene and/or its metabolites.

Table 9.3: Mean covalent binding of radioactivity from ^{14}C-paradichlorobenzene to rat tissue proteins *in vivo* (nmol/mg protein; N = 6 animals/group)

Tissue	Hours post-treatment	Control	Phenobarbitone	Phenobarbitone + SKF-525-A
Liver	6	0.021	0.012**	0.006^
	24	0.010	0.012	ND
Kidney	6	0.0091	ND	ND
	24	0.0032	ND	ND

**p<0.01 *vs.* controls ^p<0.01 *vs.* phenobarbitone alone. ND = No data

Rimington and Zeigler (1963) studied the porphyrinogenic effects of paradichlorobenzene in male albino rats (150–250 g bw), given at various doses by gastric intubation at 100–200 mg/mL in liquid paraffin or 1% cellofas (the chemical identity of the latter vehicle is unknown). The dose of the test chemical was increased gradually to 770-850 mg/kg bw/d, a level that caused high urinary porphyrin excretion yet few deaths. Animals received this dose for 5 days prior to determination of porphyrins and their precursors in the urine and liver.

Results

Treated rats displayed bodyweight loss and inappetence, and 1 animal became paralysed on its left side. Highly porphyric rats became extremely weak, ataxic, and showed clonic contractions. Interpretation of results is complicated by an apparent vehicle effect, which may have arisen because of differences in gastric absorption of the test chemical from cellofas and paraffin. The sole control group received cellofas; by comparison, urinary coporphyrin III and uroporphyrin excretion increased by *ca* 2-fold and 10-fold in rats dosed with paradichlorobenzene in the same vehicle. However, there was little or no effect on excretion of porphobilinogen and δ-aminolaevulinic acid. By comparison with the cellofas-treated controls, animals receiving l,4-dichlorobenzene in paraffin excreted 10 to 500 times the amount of all of the index chemicals.

A treatment-related increase of an order of magnitude in copro- and uroporphyrin levels occurred in rats given the test chemical in paraffin. There were no effects on hepatic coproporphyrin concentration, catalase activity or glutathione content. Histological examination revealed degeneration of individual liver cells without actual necrosis, or small areas of focal necrosis in the central, midzonal and periportal regions.

Table 9.4: Mean peak urinary porphyrin and precursor excretion levels in rats

Parameter	Control (Cellofas vehicle)	paradichlorobenzene 850 mg/kg bw/d (Cellofas vehicle)	paradichlorobenzene 770 mg/kg bw/d (Paraffin vehicle)
Urinary excretion			
Coproporphyrin (µg/d)	4.3 – 6.8	15	62
Uroporphyrin (µg/d)	0.1 – 0.3	0.83	11
Porphobilinogen (µg/d)	2.5 – 6.5	7.6	1328
δ-aminolaevulinic acid (µg/d)	39 - 52	49	437
Liver			
Coproporphyrin conc. (µg/100 g)	4.5	ND	5.1
Protoporphyrin conc. (µg/100 g)	9.7	ND	61
Uroporphyrin conc. (µg/100 g)	1.3	ND	60
Catalase activity (meq/mg tissue)	0.85	ND	0.88

Umemura *et al* (1996) administered paradichlorobenzene (Wako Chemical Co, Osaka, Japan; >98% purity) to male B6C3F1 mice (7 weeks old; from Japan SLC Inc, Shizuoka, Japan) by gastric intubation at 600, 1000 or 1800 mg/kg bw (5% w/v in corn oil). Controls received vehicle alone. One to 7 days post-treatment, animals were injected with 5-bromo-2'-deoxy-uridine (BrdU) and killed 2 hours later. Acute hepatic injury was assessed by measurement of serum ALT activity and histological examination. The rate of cellular replication was estimated from the extent of BrdU incorporation into hepatocytes.

Results

Results were presented in pictorial form unsuitable for quantitative analysis. In mice killed two days post-treatment, the most sensitive indicator of physiological disturbance was BrdU labelling index, which showed a clear dose-response, rising from *ca* 0.2% at 600 mg/kg to 2.5% at 1800 mg/kg. Statistical significance ($p<0.05$) vs. control (in which there was little or no labelling) was attained at 1000 and 1800 mg/kg. At the latter dose serum ALT activity increased to 4.5 X the control value ($p<0.05$), but despite mild centrilobular hepatocyte swelling there was little necrosis.

When the time course of responses to a 1800 mg/kg dose was followed over 7 days, labelling index was at control level 24 hours post-treatment, peaked at *ca* 2.0% on days 2 and 3 (indicating enhanced cell replication) and returned to baseline by day 7. ALT activity rose above and declined to baseline in parallel to labelling index but there was little if any hepatocellular necrosis.

Therefore, it appears that in mice at relatively high acute doses, paradichlorobenzene exerts a mitogenic effect, stimulating liver cell proliferation in response to non-lethal cellular injury or dysfunction. The study authors suggested that mitogenesis was the mechanism by which the test chemical caused hepatocellular carcinomas in the NTP (1987) 2-year mouse study.

Umemura *et al* (2000) (Assessed by EU, 2004) found no evidence of oxygen adducts with DNA in the kidneys of male F344 rats treated with paradichlorobenzene in corn oil by gavage at 0 or 300 mg/kg bw/d, 5 d/wk for 13 wk. A significant response to the positive control, potassium bromate demonstrated the sensitivity of the test system.

10 Other Studies

(Suzuki *et al*, 1991) (Assessed by US EPA, 2006) found no effects in a subchronic immunotoxicity study of inhaled paradichlorobenzene in guinea pigs. Groups of 10 male Hartley guinea pigs were exposed to concentrations of 0, 2 or 50 ppm for 12 weeks (exposure schedule not specified). The animals were sensitized with ovalbumin twice during the exposure period (4 and 8 weeks after exposure commencement) to evaluate effects on immunoglobulin [Ig]E, IgG, and IgM antibody production. IgE antibody titres (passive cutaneous anaphylaxis test) and IgG and IgM antibody titres (determined by ELISA) against ovalbumin in serum collected 1 and 2 weeks after the first sensitization and 1, 2, and 4 weeks after the second sensitization, showed no significant differences between the exposed and control groups. The passive cutaneous anaphylaxis test was also conducted with antiserum from the 50 ppm exposure group (collected on the above schedule) to determine if IgE antibodies were produced against paradichlorobenzene. No antibodies against the compound were detected. Active systemic anaphylaxis was also evaluated in the 0 and 50 ppm exposure groups. An antigen mixture of paradichlorobenzene and guinea pig serum albumin did not cause an anaphylactic reaction when injected intravenously in the animals 14 days after the last exposure. There were no exposure-related effects on other study endpoints, including body weight, haematology (including total and differential leukocyte counts), and absolute and relative weights of selected organs (thymus, spleen, liver, kidneys, lungs, and heart), indicating that 50 ppm was the subchronic NOAEC for immunological and other systemic effects in guinea pigs.

11 Clinical Case Studies

Campbell and Davidson (1970) presented a case report of a 21-year old pregnant woman who developed haemolytic anaemia as a result of ingesting toilet air-freshener blocks that contained paradichlorobenzene. These had been consumed at the rate of 1 or 2 per week throughout the 38-week duration of her pregnancy. No methaemoglobin or sulfhaemoglobin was detected in the plasma, there was no evidence of hepatic dysfunction, BUN and urinalysis was normal and there was no haemosiderinuria. Following an induced birth, the infant had no clinical or haematological abnormalities. The patient's illness resolved within six weeks of delivery. [Assuming a bodyweight of 60 kg and that each block contained 20 g of paradichlorobenzene, the achieved dose would have been *ca* 20 000 to 40 000 mg ÷ 7 d ÷ 60 kg = 47 to 95 mg/kg bw/d.]

Cotter (1953) examined 4 patients who had been exposed (principally via inhalation) to paradichlorobenzene in domestic and working environments for between 3 months and 2 years. Cirrhosis of the liver and/or jaundice had developed each individual and 2 of them died from liver failure. Some or all patients also displayed splenomegaly, anaemia, petechial haemorrhages, haematuria and casts in the urine and elevated BUN. The latter findings are suggestive of renal toxicity. A further individual who used a paradichlorobenzene-based insecticide in the home presented with periorbital swelling, intense headache and profuse rhinitis which resolved within 24 hours.

Hallowell (1959) reported a case of acute haemolytic anaemia in a 3 year old boy who had played with paradichlorobenzene de-mothing crystals in his home. The child was presumed to have ingested the chemical but only dermal contact was witnessed. Inhalation exposure is also likely to have occurred.

In addition to methaemoglobin, the child's urine contained 'traces' of 2,5-dichloroquinol and at least 2 other phenols, but 2,5-dichlorophenol was not found.

Hollingsworth *et al* (1956) summarised occupational health experience with paradichlorobenzene at a large industrial plant, where the chemical was manufactured and handled. Airborne concentrations ranged between 10 and 725 ppm, with average values of 45–105 ppm under most conditions. The threshold of odour detection was 15-30 ppm, with the odour being perceived as strong at 30-60 ppm. Fumes and vapour concentrations of 80-160 ppm were painful or irritating to the eyes and nose and higher levels could not be tolerated. Solid particles of paradichlorobenzene were painful to the eyes but caused 'negligible' irritation to intact, uncovered skin, although warm fumes or strong solutions were slightly irritating upon prolonged or repeated dermal contact. No cataracts, haematological effects or abnormalities in urinalysis were found in exposed employees.

Miyai *et al* (1988) (Assessed by WHO, 1991) recorded nervous system dysfunction (limb and truncal ataxia, dysarthia, hyporeflexia, hypotonus and mild proximal limb weakness) in a 25-year old woman exposed to paradichlorobenzene for 6 years through extensive use of mothballs in her bedclothes and wardrobe. She developed difficulty in speech and gait. Exposure was not quantified.

Morita and Ohi (1975): Adipose tissue samples from a group of 34 Tokyo residents aged 13–80 years contained paradichlorobenzene at an average of 2.3 µg/g (range: 0.2-12 µg/g). The average concentration of paradichlorobenzene in blood from an additional 6 individuals was 9.5 ng/mL (range: 4-16 ng/mL). paradichlorobenzene levels in outdoor air samples from 6 locations in central and suburban Tokyo were 1.5-4.2 µg/m^3. Much higher concentrations (respectively 105, 315 and 1700 µg/m^3) were detected indoors in a bedroom, closet and wardrobe. The study authors did not comment as to whether any donors had been exposed occupationally, or correlate paradichlorobenzene levels in fat and blood with those in the atmosphere to which donors had been exposed.

Nalbandian and Pearce (1965) reported a case involving a man exposed to paradichlorobenzene crystals by sitting in a chair that had been treated with the chemical. While seated, he had experienced dyspnoea, which was followed 24-48 hours later by swelling of the hands and feet and development of petechiae and purpurea on his arms and legs. A subsequent episode of acute glomerulonephritis (seen as proteinuria and elevated BUN) developed from 6 days post-exposure. Serum antibodies to paradichlorobenzene were present and persisted for at least 5 months post-exposure. The study authors attributed renal injury to allergic purpurea, rather than to any direct toxic effect of the chemical.

Weller and Crellin (1953) reported a case of pulmonary granulomatosis in a woman with a 12–15 year history of heavy exposure to vapour from paradichlorobenzene crystals used in her residence for moth control. Biopsied lung tissue contained numerous small granular lesions, fibrous tissue proliferation and giant cells centred around prominent plate-like crystals physically similar to those of paradichlorobenzene. Some of the giant cells contained similar crystals within their cytoplasm. Surrounding lung parenchyma was distorted due to fibrosis, thickened alveolar walls and heavy infiltration by lymphocytes and mononuclear phagocytes. There was slight to moderate thickening of the muscular walls of the small arteries and local fibrous thickening of the pleura.

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APPENDIX 1

Clinical biochemistry, haematology and urinalysis parameters

Clinical biochemistry	Haematology	Urinalysis
albumin	blood smear	AAP~
AP*	clotting time	appearance
ALT*	haemoglobin*	bilirubin*~
bilirubin (total)*	haematocrit~	copro- & uroporphyrins*
BUN*	MCH	creatine*
calcium	MCHC	glucose*~
chloride	MCV*	ketones*~
cholesterol (total)*	PCV*	LDH~
cholinesterase activity	platelet count*	NAG~
corticosterone~	prothrombin time	occult blood*~
CPK	RBC count*	pH*~
creatinine (blood)~	reticulocyte count*	protein*~
GGT*	WBC total & differential count*	specific gravity*
globulin*		sediment (microscopic)~
glucose (blood)*		testosterone~
LDH		urobilinogen~
phosphorus		volume*~
potassium		
protein (total)*		
sodium		
testosterone~		
triglycerides*		

*Analysed in US NTP (1987) 13-week studies in mice and rats
~Analysed in Bomhard *et al* (1988)

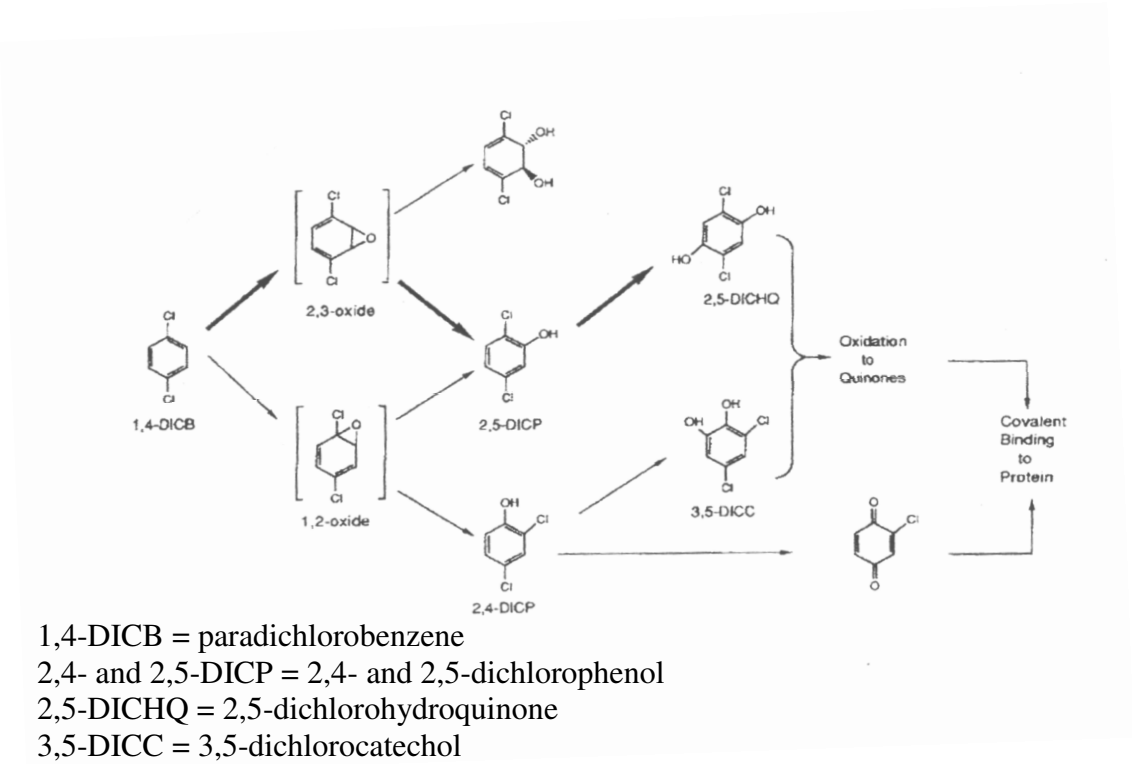
Organs for weight determination and histopathological examination

Organs weighed	Tissues examined		
adrenals	adrenals*^#~	heart*^#~	prostate^#~
brain*	aorta*#~	ileum*^#~	rectum~
gonads*	blood smear	jejunum#~	salivary gland*^#~
heart*	bone*^#	kidneys*^#~	seminal vesicle#
kidneys*	bone marrow*^#	lachrymal gland	skin^~
liver*	brain*^#~	larynx*#	spinal cord (cervical,
lung*	caecum*#~	liver*^#~	thoracic, lumbar*#~)
spleen*	cervix#	lungs*^#~	spleen*^#~
thymus*	colon*^#~	lymph nodes*^#~	sternum*~
thyroid	duodenum*#~	mammary gland*^#	stomach*^#~
(w/parathyroid)	epididymis#~	muscle (smooth)	testes*^#~
uterus*	eyes*^#~	muscle (skeletal)*#~	thymus*^#~
	eyes (optic nerve)	nerve (peripheral)*	thyroid#
	gall bladder^~	nerve (sciatic)#~	(w/parathyroid)*^~
	Harderian glands#	oesophagus*^#~	trachea*^#~
	head (nasal cavity*#,	ovaries*^#~	urinary bladder*^#~
	para- nasal sinus*,	pancreas*^#~	uterus*^#~
	tongue, oral cavity,	pinna*	vagina
	naso- pharynx, inner	pituitary*^#~	Zymbal's gland#
	ear*)		gross lesions*^

*Analysed in US NTP (1987) 13-week studies in mice and rats
^ Analysed in US NTP (1987) 24-month studies in mice and rats
#Analysed in Loeser and Litchfield (1983) 13- and 18-month studies in mice and rats
~Analysed in Naylor *et al* (1996) 12-month study in dogs

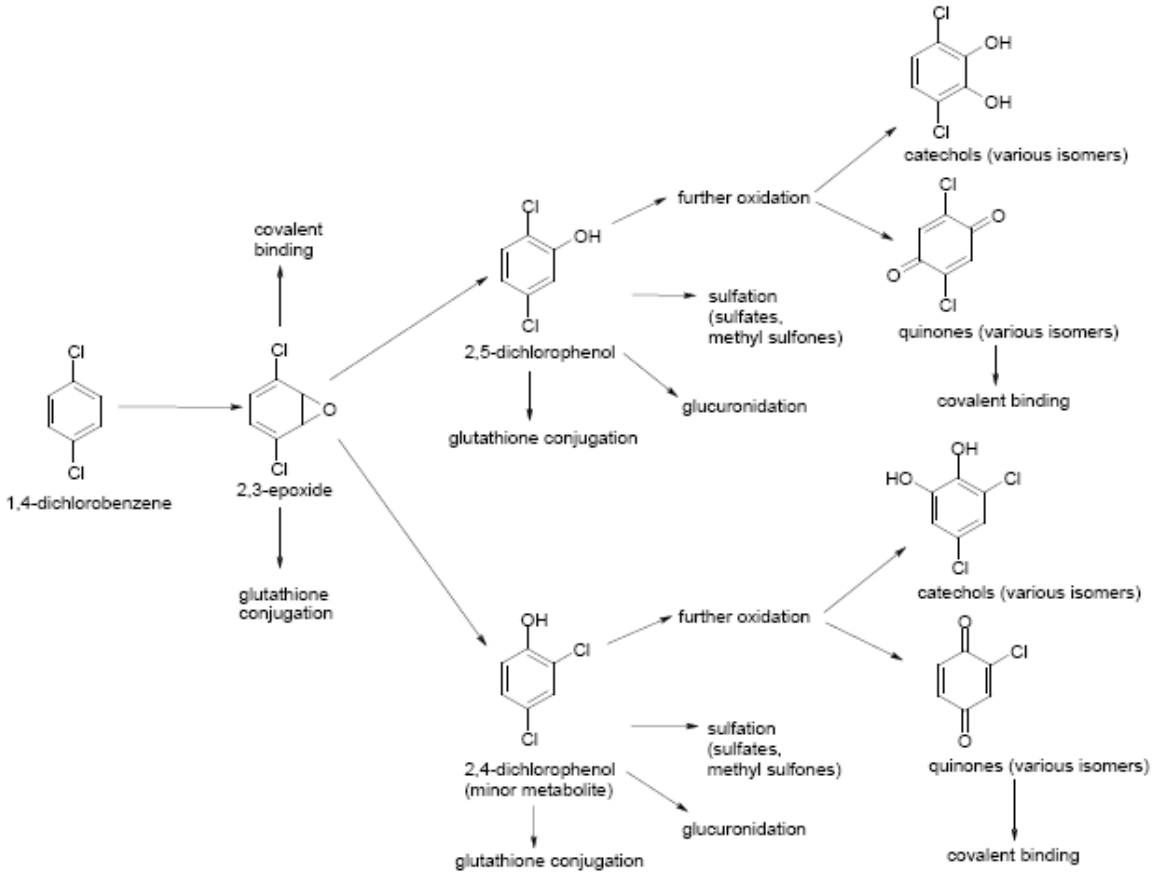
APPENDIX II

Postulated metabolic pathway of paradichlorobenzene in rat hepatic microsomes (den Besten *et al* (1992)



1,4-DICB = paradichlorobenzene
2,4- and 2,5-DICP = 2,4- and 2,5-dichlorophenol
2,5-DICHQ = 2,5-dichlorohydroquinone
3,5-DICC = 3,5-dichlorocatechol

Postulated metabolic pathway of paradichlorobenzene *in vivo* (US EPA, 2006)



Note: In humans, metabolism proceeds predominantly via the 2,3-epoxide (shown in this figure). In rats and mice, metabolism proceeds via the 1,2- and 2,3-epxoide (Muller, 2002).

Attachment 3

Dietary Exposure Assessment Report

Executive Summary

Dietary exposures to paradichlorobenzene (PDCB) were calculated for the Australian and New Zealand populations aged 2 years and above and 15 years and above, respectively, and for the population sub-group of Australian children aged 2-6 years.

The food consumption data used were from the 1995 Australian National Nutrition Survey (NNS) and the 1997 New Zealand NNS. The concentration data for PDCB in honey was from the Application and was the proposed ERL for PDCB of 0.1 mg/kg.

For Australian consumers of honey, the estimated mean PDCB dietary exposure for the population aged 2 years and above was 1.3 µg/day. Estimated 90th percentile dietary exposures to PDCB were 2.9 µg/day. Seventeen percent (17%) of the Australian population aged 2 years and above were consumers of honey and products and honey in mixed dishes.

For New Zealand consumers of honey, the estimated mean exposures to PDCB for the population aged 15 years and above was 1.7 µg/day which is higher than for than for Australians aged 2 years and above (1.3 µg/day). Estimated 90th percentile dietary exposure to PDCB was approximately 3.9 µg/day. As for Australia, approximately 17% of the New Zealand population were reported as being consumers of honey and products and honey in mixed dishes.

For Australian children 2-6 years, the estimated dietary exposure to PDCB for honey consumers (0.7 µg/day) was less than that for Australians aged 2 years and above (1.3 µg/day) and New Zealanders aged 15 years and above (1.7 µg/day). Ninetieth (90th) percentile exposures were also lower for Australian children aged 2-6 years (1.6 µg/day).

In order to determine whether the level of estimated dietary exposures to PDCB will be of concern to public health and safety, exposures were compared to a tolerable daily intake (TDI) of 0.1 mg/kg bw/day which was provisionally established by FSANZ. Estimated dietary exposures to PDCB were well below the tolerable daily intake (TDI) for all population groups investigated (<0.1%).

1. Dietary modelling

1.1 What is dietary modelling?

Dietary modelling is a tool used to estimate exposures to food chemicals from the diet as part of the risk assessment process. To estimate dietary exposure to food chemicals records of what foods people have eaten are required along with information on how much of the chemical is in each food. The accuracy of these exposure estimates depends on the quality of the data used in the dietary models. Sometimes, not all of the data required are available or there is uncertainty about the accuracy so assumptions are made, either about the foods eaten or about chemical levels, based on previous knowledge and experience.

The models are generally set up according to international conventions for food chemical exposure estimates, however, each modelling process requires decisions to be made about how to set the model up and what assumptions to make; a different decision may result in a different answer. Therefore, FSANZ documents carefully all such decisions and model assumptions to enable the results to be understood in the context of the data available and so that risk managers can make informed decisions.

1.2 Population groups assessed

The dietary exposure assessments were conducted for both Australian (2 years and above) and New Zealand (15 years and above) populations as well as for Australian children aged 2-6 years. Dietary exposure assessments were conducted for the whole population as a proxy for lifetime exposure. An exposure assessment was conducted for Australian children aged 2-6 years because children generally have higher dietary exposures due to their smaller body weight, and they consume more food per kilogram of body weight compared to adults. It is important to note that, while children aged 2-6 years have been assessed as a separate group, this group has also been assessed in the dietary exposure assessment for the Australian population aged
2 years and above.

The dietary exposure assessment was conducted using dietary modelling techniques that combine food consumption data with food chemical concentration data to estimate the exposure to PDCB from the diet. The dietary exposure assessment was conducted using FSANZ's dietary modelling computer program, DIAMOND.

Dietary exposure = food chemical concentration x food consumption

The exposure was estimated by combining usual patterns of food consumption, as derived from national nutrition survey (NNS) data for Australia and New Zealand, with the proposed levels of PDCB in honey and products and dishes containing honey. The overall approach to the dietary exposure assessment is shown in Figure 1.

1.3 Dietary Modelling Approach for consideration of an ERL for PDCB in Honey

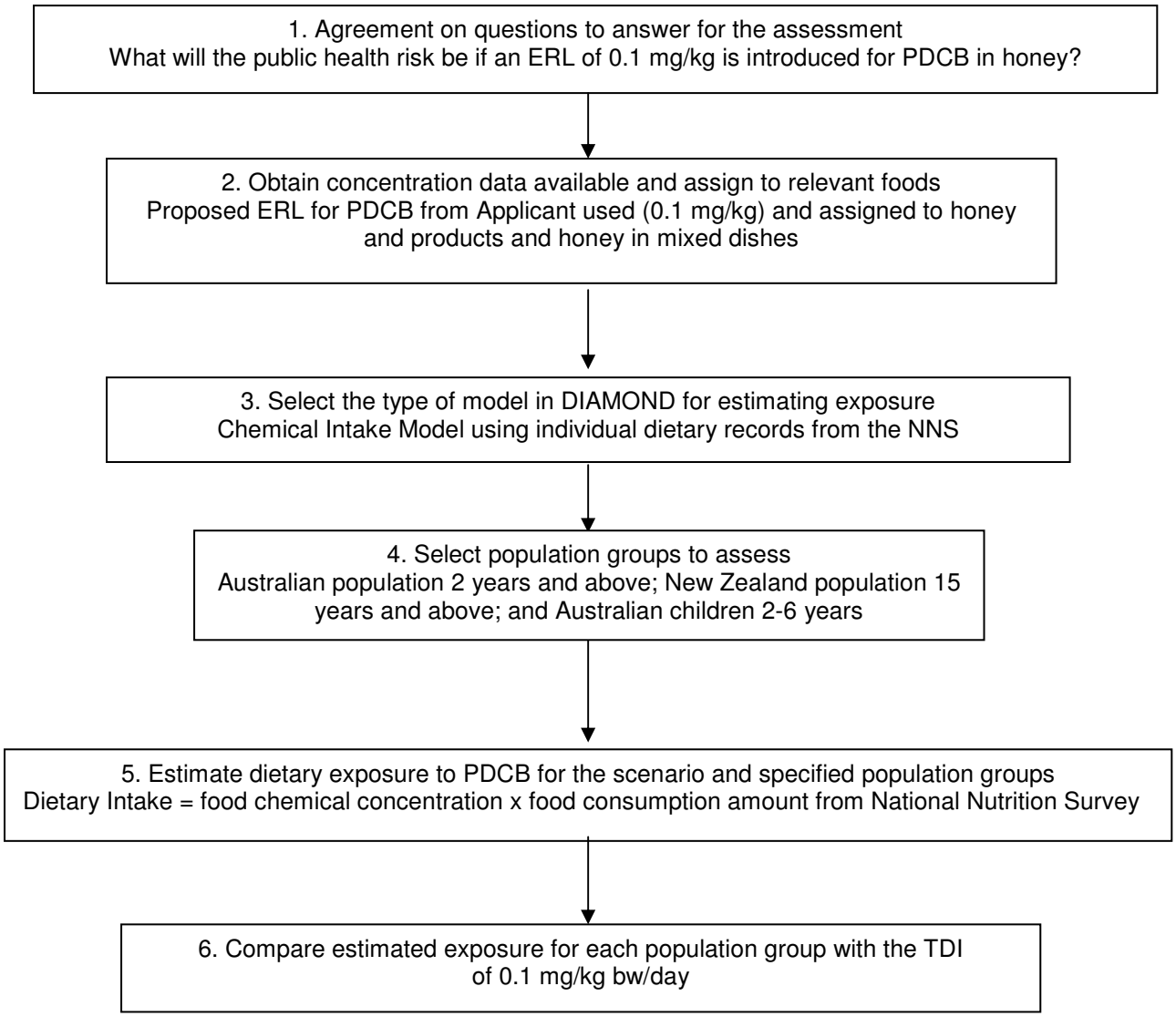


Figure 1: Dietary Modelling approach used for A602 – PDCB in Honey

1.4 Dietary survey data

DIAMOND contains dietary survey data for both Australia and New Zealand; the 1995 NNS from Australia that surveyed 13,858 people aged 2 years and above, and the 1997 New Zealand NNS that surveyed 4,636 people aged 15 years and above. Both of the NNSs used a 24-hour food recall methodology. It is recognised that these survey data have several limitations. For a complete list of limitations see Section 4. Limitations of the dietary modelling).

1.4.1 Additional food consumption data or other relevant data

No further information was required or identified for the purpose of refining the dietary exposure estimates for this Application.

1.4.2 PDCB concentration levels

The levels of PDCB in foods that were used in the dietary exposure assessment were from the Applicant's proposed ERL of 0.1 mg/kg. There are no other foods with ERLs or MRLs for PDCB in Australia or New Zealand. Additionally, no MRL or ERL has been established for PDCB in honey as part of Codex or European Union (EU) legislation.

Concentrations of PDCB were assigned to food groups using DIAMOND food classification codes. These codes are based on the raw commodity classifications.

1.5 How were the estimated dietary exposures calculated?

A detailed explanation of how the estimated dietary exposures are calculated can be found in Appendix 1.

2. Assumptions in the dietary modelling

The aim of the dietary exposure assessment was to provide a realistic estimate of dietary exposure. However, where significant uncertainties in the data existed, conservative assumptions were generally used to ensure that the dietary exposure assessment did not underestimate exposure.

The assumptions made in the dietary exposure assessments are listed below, broken down into several categories.

Concentration data

- where a food or food group has a zero concentration of PDCB, it was not included in the dietary exposure assessment;
- no other foods except honey contain PDCB; and
- where a food has a specified PDCB concentration, this concentration is carried over to mixed foods where honey has been used as an ingredient e.g. prawn stir fry in honey sauce.

Consumption data

- consumption of foods as recorded in the NNS represents current food consumption patterns; and
- there is no consumption of PDCB through consumption of products such as royal jelly, beeswax or honeycomb (since no respondents in the NNS reported consumption of these products).

Consumer behaviour

- consumers always select honey and honey products as well as honey in dishes that contain PDCB.

General

- there are no reductions in PDCB concentrations from food preparation or due to cooking; and
- there is no contribution to PDCB exposure through the use of complementary medicines (Australia) or dietary supplements (New Zealand).

These assumptions are likely to lead to a conservative estimate for PDCB dietary exposure.

3. Results

3.1 Estimated dietary exposures to PDCB

The estimated dietary exposures for PDCB are shown in Table 1 (full results in Table A2.1 in Appendix 2). Seventeen per cent of Australians 2 years and above were consumers of honey and products and dishes containing honey. For consumers of honey aged 2 years and above, the estimated mean dietary exposure to PDCB was approximately 1.3 µg/day. The estimated 90th percentile dietary exposures were 2.9 µg/day for Australian consumers aged 2 years and above and 1.6 µg/day for Australian children aged 2-6 years.

Similar to Australia, 17% of New Zealanders 15 years and above were consumers of honey and products and dishes containing honey. The estimated mean dietary exposure to PDCB for consumers of honey was 1.7 µg/day. Estimated 90th percentile dietary exposure was approximately 3.9 µg/day.

Table 1: Estimated dietary exposures to PDCB for Australian and New Zealand population groups

Country	**Population Group**	**Estimated Dietary Exposure for Consumers♦ of PDCB (µg/day)**	
		Mean	90th Percentile
Australia	2 years and above	1.29	2.86
	2-6 years	0.73	1.56
New Zealand	15 years and above	1.70	3.87

♦ Consumers – This only includes the people who have consumed a food containing PDCB.

4. Limitations of the dietary modelling

Dietary modelling based on 1995 or 1997 NNS food consumption data provides the best estimate of actual consumption of a food and the resulting estimated dietary exposure to a chemical for the population. However, it should be noted that the NNS data do have limitations. These limitations relate to the age of the data and the changes in eating patterns that may have occurred since the data were collected. Generally, consumption of staple foods such as fruit, vegetables, meat, dairy products and cereal products, which make up the majority of most people's diet, is unlikely to have changed markedly since 1995/1997 (Cook *et al*., 2001a; Cook *et al*., 2001b).

However, there is uncertainty associated with the consumption of foods that may have changed in consumption since 1995/1997, or that have been introduced to the market since 1995/1997.

A limitation of estimating dietary exposure over a period of time associated with the dietary modelling is that only 24-hour dietary survey data were available, and these tend to over-estimate habitual food consumption amounts for high consumers. Therefore, predicted high percentile dietary exposures are likely to be higher than actual dietary exposures for high consumers over a lifetime, in particular when the food chemical is found in occasionally consumed foods, such as honey. For this reason 90th percentile dietary exposure estimates were used in this assessment to assess potential risks for high consumers of PDCB from honey over a long period of time, noting that these 90^{th} percentile estimates are likely to overestimate mean daily consumption of PDCB for honey consumers over a long period of time by approximately two fold (Lambe *et al.,* 2000 and WHO, 1985).

Where the NNSs collected data on the use of complementary medicines (Australia) or dietary supplements (New Zealand), it was either not in a robust enough format to include in DIAMOND or has simply not been included in the DIAMOND program. Consequently, intakes of substances consumed via complimentary medicines or dietary supplements could not be included in the dietary exposure assessment.

While the results of NNSs can be used to describe the usual intake of groups of people, they cannot be used to describe the usual intake of an individual (Rutishauser, 2000). In particular, they cannot be used to predict how consumers will change their eating patterns as a result of an external influence such as the availability of a new type of food.

FSANZ does not apply statistical population weights to each individual in the NNSs in order to make the data representative of the population. This prevents distortion of actual food consumption amounts that may result in an unrealistic intake estimate. Maori and Pacific people were over-sampled in the 1997 New Zealand National Nutrition Survey so that statistically valid assessments could be made for these population groups. As a result, there may be bias towards these population groups in the dietary exposure assessment because population weights were not used.

5. Risk characterisation

5.1 Comparison of the estimated dietary exposures with the Tolerable Daily Intake

In order to determine if the level of dietary exposure to PDCB has the potential to be of concern to public health and safety, the estimated dietary exposures were compared to a tolerable daily intake (TDI) of 0.1 mg/kg bw/day which was provisionally established by FSANZ. Acceptable Daily Intakes (ADIs) are not maintained for agricultural and veterinary chemicals that are no longer permitted for use in agricultural practice. However, residues of certain environmentally persistent pesticides may occur as residues in agricultural commodities as a consequence of past use as is the case with PDCB in honey (Department of Health & Aging, 2006). The TDI has been used in this dietary exposure assessment to provide a guideline with which potential dietary intakes of PDCB can be compared.

5.2 Comparison of PDCB residues to non-Australian standards

There is currently no MRL or ERL for PDCB in honey as part of Codex or EU legislation. According to the Applicant, the EU Commission has set an action level of 10 ppb (0.01 mg/kg) for PDCB in pesticide legislation. This stipulates that food must not contain any pesticide which exceeds 10 ppb (0.01 mg/kg) where no specific MRL is set. In New Zealand, PDCB falls under default provisions in the Animal Products (Residue Specifications) Notice 2004 with a Maximum Permitted Limit (MPL) of 0.1 mg/kg. The maximum residue of PDCB detected in honey as indicated by the Applicant was 0.019 mg/kg, which was slightly higher than the EU action level and lower than the New Zealand MPL.

The estimated dietary exposures for PDCB, as compared to the TDI are shown in Table 2(full results in Table A3.1 in Appendix 3). For all population groups, estimated dietary exposures to PDCB were below 0.1% of the TDI for PDCB, hence there is no cause for public health and safety concern of setting an ERL for PDCB of 0.1 mg/kg with a sunset clause.

Table 2: Estimated dietary exposures to PDCB, as a percentage of the TDI

Country	Population group	Estimated dietary exposure to PDCB (%TDI*)	
		Mean consumers♦	90th percentile consumers
Australia	2 years and above	<0.1	<0.1
	2-6 years	<0.1	<0.1
New Zealand	15 years and above	<0.1	<0.1

♦ Consumers – This only includes the people who have consumed a food that contains PDCB
* TDI = 0.1 mg/kg bw/day

References

Cook, T., Rutishauser, I. and Seelig, M. (2001a) *Comparable data on food and nutrient intake and physical measurements from the 1983, 1985 and 1995 national nutrition surveys*. Australian Food and Nutrition Monitoring Unit, Commonwealth Department of Health and Aged Care, Commonwealth of Australia, Canberra.

Cook, T., Rutishauser, I. and Allsopp, R. (2001b) *The Bridging Study: comparing results from the 1983, 1985 and 1995 Australian national nutrition surveys*. Australian Food and Nutrition Monitoring Unit, Commonwealth Department of Health and Aged Care, Commonwealth of Australia, Canberra.

Department of Health & Ageing (Office of Chemical Safety). (2006) *Acceptable Daily Intakes for Agricultural and Veterinary Chemicals.* Commonwealth of Australia: Canberra.

Lambe, J., Kearney, J., Leclercrq, C., Berardi, D., Zunft,H., De Henauw, S., De Volder, M., Lamberg-Allardt, C., Karkkainen, M., Dunne, A. and Gibney, N.J. (2000) *Enhancing the capacity of food consumption surveys of short duration to estimate long term consumer-only intakes by combination with a qualitative food frequency questionnaire*. Food Additives and Contaminants, 17(3), pp. 177-187.

Rutishauser, I. (2000) *Getting it right: how to use the data from the 1995 National Nutrition Survey.* Commonwealth of Australia, Canberra.

WHO. (1985) *Guidelines for the Study of Dietary Intakes of Chemical Contaminants.* WHO, Geneva.

APPENDIX 1

How were the estimated dietary exposures calculated?

The DIAMOND program allows PDCB concentrations to be assigned to food groups. For the purposes of this dietary exposure assessment, honey and products and honey in mixed dishes were the only foods allocated PDCB concentrations. This is because there are currently no other permissions for PDCB in the Code and this is a new Application requesting temporary permission of PDCB in honey.

Dietary exposures for the pesticide fumigant PDCB were estimated for each individual in the NNS using his or her individual food records from the dietary survey. The DIAMOND program multiplies the specified concentration of PDCB by the amount of food that an individual consumed from that group in order to estimate the exposure to PDCB from each food. Once this has been completed for all of the foods specified to contain PDCB, the total amount of PDCB consumed from all foods is summed for each individual. Population statistics (mean and 90^{th} percentile exposures) were then derived from the individuals' ranked exposures.

Where estimated exposures are expressed as a percentage of the reference health standard (TDI), each individual's total exposure is calculated as a percentage of the TDI (using the total exposures in milligrams per kg bw), the results are then ranked, and population statistics derived.

In DIAMOND, all mixed foods have a recipe. Recipes are used to break down mixed foods into their raw commodity components (eg bread will be broken down to wheat flour, yeast, water etc). The data for consumption of the raw commodities are then used in models that assign PDCB permissions to raw commodity food codes.

Food consumption amounts for each individual take into account where each food in a classification code is consumed alone and as an ingredient in mixed foods. For example, honey in a peanut and honey bar and honey based marinade for chicken are all included in the consumption of honey.

APPENDIX 2

Complete information on dietary exposure assessment results

Table A2.1: Estimated dietary exposures to PDCB

Country	Population group	Number of consumers of PDCB	Consumers♦ as a % of total respondents#	Estimated dietary exposure to PDCB (µg/day)		
				Mean all respondents#	Mean consumers♦	90th percentile consumers♦
Australia	2 years and above	2,308	17	0.2	1.3	2.9
	2-6 years	223	23	0.2	0.7	1.6
New Zealand	15 years and above	804	17	0.3	1.7	3.9

Total number of respondents for Australia: 2 years and above = 13,858; 2-6 years = 989; New Zealand: 15 years and above = 4,636. Respondents include all members of the survey population whether or not they consumed a food containing PDCB.
♦ Consumers – This only includes the people who have consumed a food that contains PDCB.

APPENDIX 3

Complete information on risk characterisation

Table A3.1: Estimated dietary exposures to PDCB, as a percentage of TDI

Country	Population group	Number of consumers of PDCB	Consumers♦ as a % of total respondents#	Estimated dietary exposure to PDCB (%TDI)		
				Mean all respondents	Mean consumers	90th percentile consumers
Australia	2 years and above	2,308	17	0.004	0.023	0.054
	2-6 years	223	23	0.009	0.039	0.091
New Zealand	15 years and above	804	17	0.004	0.025	0.053

Total number of respondents for Australia: 2 years and above = 13,858; 2-6 years = 989; New Zealand: 15 years and above = 4,636. Respondents include all members of the survey population whether or not they consumed a food containing PDCB.

♦ Consumers – This only includes the people who have consumed a food that contains PDCB.

* TDI = 0.1 mg/kg bw/day

Attachment 4

Summary of Other Correspondence

Although FSANZ decided not to invite public submissions prior to making a Draft Assessment, a number of organisations provided comment on the Initial Assessment Report for this Application. In the interests of transparency, the issues raised in this correspondence have been considered by FSANZ in preparing this Draft Assessment Report.

The Australian Food and Grocery Council supported the incorporation of an ERL of 0.1 mg/kg for paradichlorobenzene in honey for a period of five years.

The Food Technology Association of Australia Inc supported the incorporation of an ERL of 0.1 mg/kg for paradichlorobenzene in honey for a period of five years.

The NSW Food Authority supported progression of the Application to Draft Assessment, subject to consideration of specific issues listed below and did not express a preference for a specific option.

The Queensland Department of Primary Industries and Fisheries, through Queensland Health, provided information for consideration by FSANZ (see below) and, conditionally, did not support the incorporation of an ERL of 0.1 mg/kg for paradichlorobenzene in honey.

Correspondent	Issue	How FSANZ has addressed
NSW Food Authority	Seeks guidance on establishing the limit at 0.1 mg/kg.	This has been assessed in the Report and is based on a limit that is achievable, safe and consistent with the limit that is being applied in New Zealand, and which would therefore apply to honey exported from New Zealand to Australia.
	Does not understand how setting an ERL would address the industry problem arising from detections above 0.01 mg/kg.	Incorporating an ERL of 0.1 mg/kg would allow the sale of honey in Australia containing residues up to 0.1 mg/kg. It would have limited relevance to export markets as honey exporters would need to meet the requirements of export markets, including any specific limits.
	Solution must allow incidental residues to be distinguished from unregistered use.	FSANZ is unaware of any mechanism for achieving this, particularly given the limited data that is available.
	Requests FSANZ investigate active monitoring of residues in honey e.g. through National Residue Survey (NRS), industry testing, including disclosure of test results undertaken by honey producers.	FSANZ understands that the industry has already organised NRS testing and these would be published when available.

Correspondent	Issue	How FSANZ has addressed
NSW Food Authority	Requests FSANZ investigate in cooperation with necessary parties trace back investigations into source residues identified by monitoring, including illegal use.	FSANZ is unable to undertake statutory investigations or compliance activities such as performing trace back investigations. However, there are national forums where these issues can be discussed and FSANZ would be prepared to assist in these discussions, if necessary.
	Requests FSANZ investigate promotion of strategies to remove paradichlorobenzene and report on progress.	In accordance with the industry strategy, FSANZ understands that a number of actions have already been instituted by the industry.
	Requests FSANZ investigate review/withdrawal of the ERL if continuing illegal use was found to be a significant source of residues.	In accordance with the FSANZ Act, FSANZ would review the ERL if States or Territories advised FSANZ that the ERL was promoting misuse.
Queensland Department of Primary Industries and Fisheries	Paradichlorobenzene (PDCB) was approved in Queensland for use on beehives, equipment, etc for control of wax moths as an off-label permit and was recently withdrawn. No MRL was established for PDCB in honey, which suggests that legitimate use did not produce detectable residues in the honey. It is still to be substantiated whether paradichlorobenzene is an environmental contaminant.	FSANZ notes the historical approved use and its recent revocation. There are data available which suggest detectable residues may occur in honey from hives treated with paradichlorobenzene. In addition, the Applicant has provided data indicating that beeswax contains residues of PDCB and that as a result residues are occurring in honey.
	PDCB is a compound with a relatively high vapour pressure and if used as a fumigant might be expected to evaporate reasonably quickly. i.e. unlikely to be persistent in the environment.	FSANZ agrees. However, data are available which suggest that residues persist in the wax and honeycomb of the hive.
	If ongoing residues of PDCB continue to occur in honey over time, at some point a default assumption may be that of ongoing chemical misuse, unless data is provided to show that PDCB is an environmental contaminant or that the levels that are present in beeswax are in the production system. If residues in honey arise from chemical misuse an ERL is inappropriate.	FSANZ agrees that at some point ongoing detections would indicate misuse. FSANZ also agrees that an ERL should not promote misuse. FSANZ would be prepared to review the ERL if compliance agencies advised FSANZ that the ERL was promoting misuse. There are data which suggest that residues could have resulted from the legitimate historical use of paradichlorobenzene.

Correspondent	Issue	How FSANZ has addressed
Queensland Department of Primary Industries and Fisheries	As to the appropriateness of a five year timeframe for an ERL standard, the Application refers to the implementation of the industry strategy for phasing out residues. Is this industry strategy documented?	The industry strategy is attached.
	If residues in honey arise from the continued use of contaminated beeswax an ERL is inappropriate. The wax is part of the food production system.	FSANZ generally agrees but this paradichlorobenzene content may not be known until honey is produced. An ERL would allow the implicated honey to be sold while corrective action is implemented on implicated hives. Not incorporating an ERL would mean that implicated honey would have to be disposed of other than through the food supply.
	If an ERL is to be established the proposed limit may need review as to whether 0.1 mg/kg or 0.01 mg/kg is more appropriate.	A limit of 0.01 mg/kg would be insufficient to account for residues being detected, whereas a limit of 0.1 mg/kg would be sufficient and would be consistent with the limit being applied to honey from New Zealand.

Attachment 5

Industry Paradichlorobenzene Strategy

AUSTRALIAN HONEY BEE INDUSTRY COUNCIL
Address: Suite 4, Level 6, 99 Elizabeth Street, Sydney NSW 2000 Telephone: 02 9221 0911
Mailing Address: PO Box R838, Royal Exchange NSW 1225 Facsimile: 02 9221 0922
Email Address: ahbic@honeybee.org.au Web Site: www.honeybee.org.au
ABN: 63 939 614 424

6 March 07

Food Safety & Prevention of Residues Committee
PDB Strategy

1. **Literature Review**
   a. Review current data and literature associated with PDB
   b. Prepare a scientific summary report

2. **FSANZ Standard Application 1.4.1**
   a. Complete the FSANZ General Application Form & required documentation based on NZ precedent

3. **Residue Data Acquisition**
   a. Consolidate test results and time degradation trial results

4. **Technical Trials**
   a. Conduct laboratory trials to investigate evaporation techniques that may reduce PDB levels in honey
   b. Determine the influence of wax filtration on PDB levels in processed honey
   c. Confirm the absence of residues associated with phosphine gas treatment

5. **PDB Industry Brochure**
   a. From literature, provide valid mechanisms and practices that beekeepers can implement to assist in the elimination of PDB residues (eg. Airing frames, extraction times, comb replacement)

6. **Chemical Use Education**
   a. Review options and implement a strategy to educate beekeepers on the use of PDB alternatives (eg. Cold rooms, light, phosphine gas, IPM)
   b. Provide a Brochure with State based information for Beekeepers on PDB alternatives, including phosphine gas
   c. Provide a Brochure on appropriate and registered chemical use in the beekeeping industry
   d. Develop a mechanism to ensure the provision of beekeeper friendly chemical user courses for phosphine gas
   e. Provide information to quality system auditors (eg. BQUAL) for assessing beekeeper chemical use compliance

f. Review and recommend an appropriate national Vendor Declaration that is more specific on chemical use
g. Request a copy of QLD DPI advice statement to beekeepers on PDB use
h. Identify potential sale outlets for PDB and inform them in writing of the requirement to cease sale of PDB for use as wax moth control

7. Communication

a. Prepare a Draft Media Release
b. Prepare a Industry Statement

Ben McKee
FSPRC Chair

Attachment 6

Paradichlorobenzene Residues in Honey

Summary

The available information indicates that residues of paradichlorobenzene (PDCB) are likely to occur in honey produced in hives that have been treated with PDCB. This is based on the virtually unlimited capacity of the wax to absorb PDCB, its retention in the wax despite any aeration, and the subsequent leaching of PDCB from the wax into honey that comes into contact with the wax.

Based on the data provided by the Applicant, only a proportion of honey samples have been found to contain low residues of PDCB and a limit of 0.1 mg/kg would be sufficient to account for the highest residues reported in honey.

The Applicant also provided further data from trials on the reduction of paradichlorobenzene residues from known individual apiaries. The data indicate that residues decline with time. The rate of decline appears to vary from apiary to apiary but in general terms the concentration appears to halve over a two month period for those apiaries with residues above 0.03 mg/kg. For those apiaries with residues initially under 0.03 mg/kg, the residues generally reduce to below detectable residues in two months.

Residue Data

The Applicant has proposed a limit of 0.1 mg/kg and initially provided monitoring data indicating that residues between 0.077 mg/kg and 0.014 mg/kg were detected in eight samples of honey. In subsequent data from 166 samples, a wider range of concentrations were reported, ranging from a maximum of 0.091 mg/kg to a minimum of 0.01 mg/kg (limit of reporting). These subsequent data included results for 115 samples in which no detectable residues were reported. In an additional twelve samples, residues were detected but these were below the limit of reporting. Tables 1, 2A and 2B below include information on the data provided by the Applicant.

The mean and median of all the reported 'positive' residues (i.e. at or above the limit of reporting) in honey are in the order of 0.04 mg/kg. The mean of all the results (including the non-detects and detections lower than the limit of reporting) is 0.012 mg/kg and the median of all these results is 0 mg/kg.

The data provided by the Applicant indicate that only a proportion of honey samples have been found to contain low level residues. Based on the range of levels reported, a limit of 0.1 mg/kg would be sufficient to account for the highest residues reported in honey. This is graphically represented in Figure 1 below.

The Applicant also provided some monitoring data indicating that waxes in implicated hives contain residues of paradichlorobenzene. The three results for residues of paradichlorobenzene in beeswax were 2.62, 3.12 and 3.22 mg/kg. Given the lipophilic nature of paradichlorobenzene, the higher residues in beeswax compared to honey are not unexpected. These data confirm that the wax in hives is a potential source of the residues in honey.

Table 1: Summary of reported residues monitoring data

Country: Australia
Commodity: Honey
Codex or National MRL: None

Residue definition: 1,4 dichlorobenzene
Limit of reporting (LOQ, mg/kg): 0.01 mg/kg

Year	No. of samples analysed	No. of residues detected	No . of residues non-detects	Number of samples in residue range (mg/kg)*						
				≤0.01	>0.01 ≤0.02	>0.02 ≤0.05	>0.05 ≤0.075	>0.075 ≤0.1	>0.1 ≤0.2	>0.5 ≤1.0
2007	8	8	0	0	3	1	3	1	0	0
2007	166	51	115	12[9]	8	13	16	2	0	0
ALL	**174**	**59**	**115**	**12**	**11**	**14**	**19**	**3**	**0**	**0**

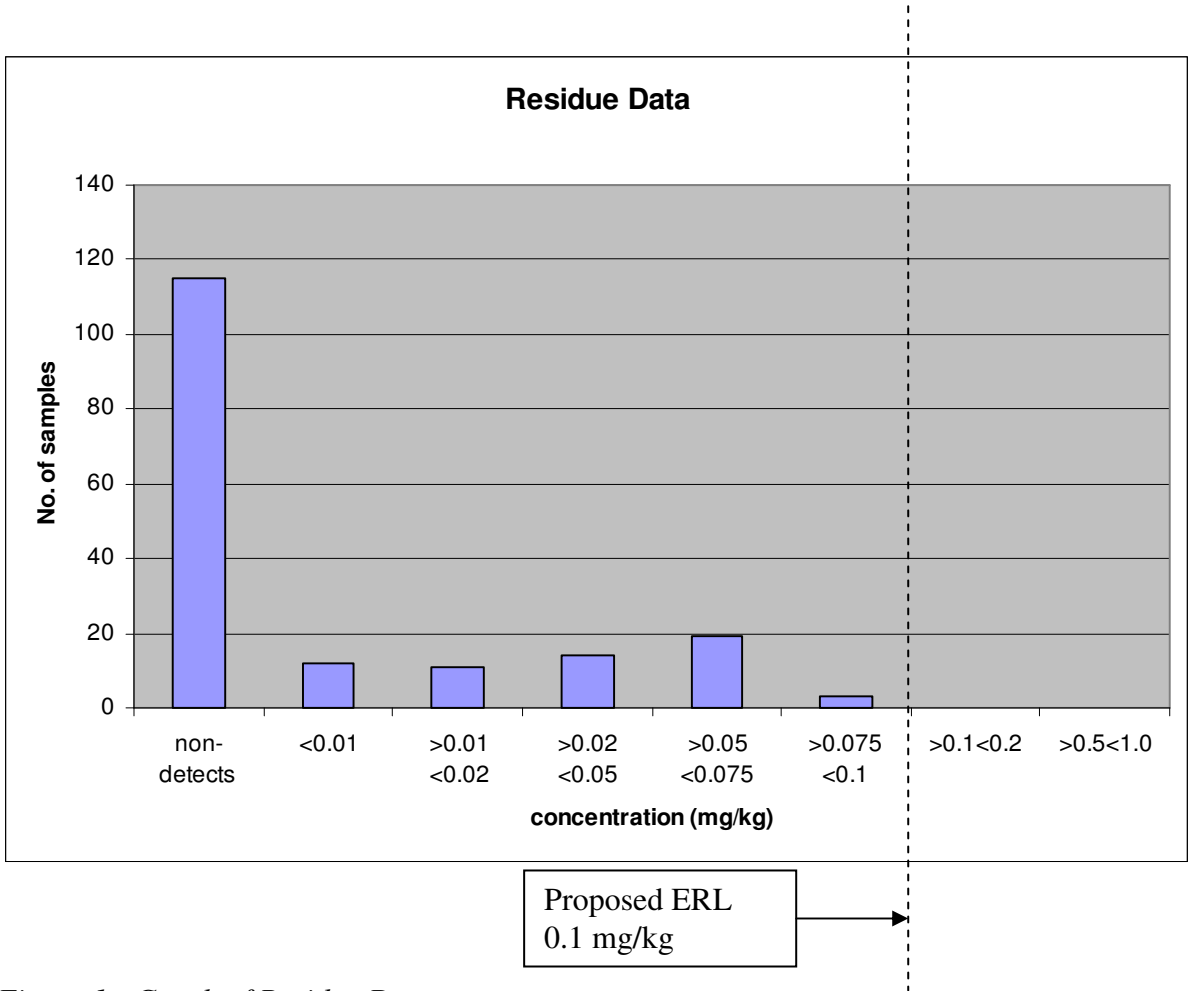


Figure 1: Graph of Residue Data

[9] Limit of Reporting is 0.01 mg/kg but detectable residues less than this limit were reported for indicative purposes.

Table 2A: Results on reported 'positive' samples

Paradichlorobenzene (ppb)	
10	
11	
12	
13	
14	
14	
16	
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59	
59	
60	
60	
61	
63	
63	
65	
67	
69	
70	
72	
74	
77	
79	
91	
43	Mean
43	Median

Table 2B: Results including non-detects

Paradichlorobenzene (ppb)	
115 non-detects[10]	
12 detects less than 10 ppb[11]	
10	
11	
12	
13	
14	
14	
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59	
60	
60	
61	
63	
63	
65	
67	
69	
70	
72	
74	
77	
79	
91	
12	Mean
0	Median

[10] (assume zero ppb)
[11] (assume 5 ppb)

The Applicant also provided further data from trials on the reduction of paradichlorobenzene residues from known individual apiaries (Table 3).

The data provided indicate that residues decline with time. While the rate of decline appears to vary from apiary to apiary, in general terms, the concentration appears to halve (one exception) over a two month period for those apiaries with residues above 0.03 mg/kg. For those apiaries with residues initially under 0.03 mg/kg, the residues generally (one exception) reduce to below detectable residues in two months.

Table 3: Positive analytical testing results for the presence of paradichlorobenzene in Australian produced honey from specific apiaries over time

	PDB levels (µg/kg)													
Sample Apiary	1	2	3	4	5	6	7	8	9	10	11	12	Total	Ave.
First Test	8	24	19	29	19	70	91	63	40	40	16	6	424.1	**35.3**
Second Test	0	0	14	0	0	35	42	59	27	22	0	0	199.0	**16.6**
Difference	8	24	5	29	19	35	49	4	13	18	16	6	225.1	**18.8**
Time (mths) between tests	2.1	2	2.1	2.1	1.9	2.5	2.5	1.7	1.9	1.7	3	3	26.5	**2.2**

Published Information

A study by Wallner[12] reported information on the characteristics of paradichlorobenzene (PDCB) absorption by beeswax, and included information on possible residues of PDCB in honey derived from wax containing PDCB. In the study it was reported that:

- PDCB is a substance with a low volatility and it is lipophilic (fat-soluble);
- honeycombs have a very large surface and have 'enormous storage capacity' for the absorption of substances like PDCB – the more PDCB is added, the more PDCB is stored in the wax;
- airtight honeycomb storage areas prevent the volatilisation of PDCB and preserve it in the honeycombs;
- the amount and speed of volatilisation are temperature dependent and at lower temperatures the volatilisation of PDCB from wax reduces. It volatilises only slightly from honey;
- even after intensive aeration, the PDCB in wax does not volatilise completely. After two to four days, 50% had volatilised from the wax and after 12 days of aeration, 8% remained in the wax;
- when storing in a cool place, high quantities of PDCB are preserved in wax and in practice it is not possible to totally remove it from the wax;
- even if honeycombs are placed into a colony for a whole season, not all the PDCB can be eliminated and it can still be detected;
- PDCB is resistant to removal during wax melting with sun or steam;
- if honey is added to cells of the treated honeycomb, PDCB penetrates slowly into the honey and, as PDCB can only evaporate from the honey surface, the majority remains in the honey.

[12] Wallner, K. 1991. The Residue of p-dichlorobenzene in Wax and Honey.

The Applicant provided FSANZ with a Swiss study by Bogdanov et al[13] which reported the presence of paradichlorobenzene (PDCB) in Swiss retail honey samples from 1997 to 2002. It was reported that:

- 30% of the Swiss honeys contained PDCB, 13% of them being above the Swiss tolerance value of 10 µg/kg (0.01 mg/kg);
- 7% of the imported honeys were found to contain PDCB;
- wax foundation and comb are possible sources of the residues in honey;
- old combs that might contain PDCB are generally recycled into foundation and PDCB is not removed during the comb recycling process or honey processing;
- while most PDCB evaporates from foundation wax under laboratory conditions (no time period stipulated), 5-10% remains trapped in the foundation wax;
- it is expected (but not proven) that PDCB would decline in foundation over time. The study authors expected this on the basis that:

    - thymol has similar volatility to PDCB (i.e. PDCB would volatilise from foundation at a similar rate to thymol); and
    - that two weeks after thymol contaminated foundation was used in a hive, only 1% of the initial concentration remained in the combs;

- approximately 0.04 mg/kg of PDCB in honey might result from honeycomb, based upon experiments with thymol (and its similar chemical and physical properties to PDCB) and laboratory experiments with the use of PDCB and comb wax. The authors stated that this hypothesis was supported by information indicating that where residues exceeded 0.01 mg/kg, the beekeepers had used PDCB for wax moth control on their honeycomb;
- long-term monitoring of Swiss beeswax from 1993 to 2000 had shown that most of the comb foundation beeswax contains PDCB ranging from 1 mg/kg to 60 mg/kg.

FSANZ was also provided with another document[14] that reported data on residues of antiparasitics and beekeeping agents in honey. It was reported that PDCB was found in Swiss and imported honey samples with all the imported samples being below the tolerance in Switzerland of 0.01 mg/kg. Approximately 30% of the Swiss honey samples were reported to contain paradichlorobenzene in excess of the 0.01 mg/kg tolerance. The document included a statement that 'while residues of up to about 0.002 mg/kg honey may result from the use of precontaminated wax, residues of more than 0.01 mg/kg indicate the use of PDCB in one's own beekeeping'. This document did not include any data or information substantiating this statement.

From the available published information, residues of PDCB are likely to occur in honey produced in hives that have been treated with PDCB. This is based on the virtually unlimited capacity of the wax to absorb PDCB, its retention in the wax despite any aeration, and the subsequent leaching of PDCB from the wax into honey that comes into contact with the wax. This has been confirmed by analyses which have shown that beeswax and honey contain PDCB.

[13] Bogdanov S, Kilchenmann V, Seiler K, Pferrerli H, Frey Th, Roux B, Wenk P, Noser J. Residues of para-dichlorobenzene in honey and beeswax. Journal of Apicultural Research, 43(1): 14-16 (2004)

[14] State Laboratory of the Canton Basel-City. 2003. Honey/residues of antiparasitics and beekeeping auxiliary agents, declaration.