
Background Information

Pentachlorophenol (CASRN 87-86-5), including its sodium salt (CASRN 131-52-2), is a chlorinated aromatic compound that is used primarily as a wood preservative in the United States. During synthesis of pentachlorophenol, several additional chlorinated molecules are formed as by-products. In addition, biomonitoring studies have found that people who are exposed to pentachlorophenol or pentachlorophenol-containing products are always exposed to the combination of pentachlorophenol and its by-products. Therefore, the candidate substance is defined as “pentachlorophenol and by-products of its synthesis” (hereafter referred to as “pentachlorophenol”). Pentachlorophenol and by-products of its synthesis have been selected as a candidate substance for the Report on Carcinogens (RoC) review based on widespread past use and current U.S. exposure and a database of studies in humans and animals specific for pentachlorophenol that are adequate for evaluating its potential carcinogenicity.

This protocol is limited to the following: (1) procedures used to prepare the cancer studies in experimental animal section of the draft RoC monograph, which will reach a level of evidence conclusion on the mixture, pentachlorophenol and by-products of its synthesis, and (2) the approach to evaluate the contribution of different grades of pentachlorophenol and individual by-products to mouse liver carcinogenicity from chronic cancer studies, which may aid in the elucidation of possible mechanisms of carcinogenicity.

Evaluation of the level of evidence of the potential carcinogenicity of pentachlorophenol and by-products of its synthesis

This section describes (1) the RoC listing criteria used in the evaluation, (2) the key scientific issues, and (3) the procedures and guidelines for each step in the animal cancer evaluation process.

RoC Listing Criteria for Evaluating Carcinogenicity from Studies in Experimental Animals

There is sufficient evidence of carcinogenicity from studies in experimental animals, which indicates there is an increased incidence of malignant and/or a combination of malignant and benign tumors (1) in multiple species or at multiple tissue sites, or (2) by multiple routes of exposure, or (3) to an unusual degree with regard to incidence, site, or type of tumor, or age at onset.

Key Scientific Questions

A primary question for the evaluation is:

- What is the level of evidence (sufficient or not sufficient) of carcinogenicity of pentachlorophenol and by-products of its synthesis from animal studies?

Secondary questions are as follows:
What are the methodological strengths and limitations of the studies?
What are the tissue sites?

Steps in the Cancer Evaluation Process
The steps for conducting the cancer evaluation are outlined below. The procedures and guidelines for conducting each step are described in Appendices A through C.

Appendix A: Selection of the literature included in the evaluation of cancer studies in experimental animals (Table A-1). Cancer studies on by-products of synthesis of pentachlorophenol from secondary sources (Table A-2).
Appendix B: Assessment of the quality of the individual animal cancer studies.
Appendix C: Assessment of the level of evidence of carcinogenicity (sufficient or inadequate) of ‘pentachlorophenol and by-products of its synthesis’ from studies in experimental animals.

Approach for the evaluation of co-exposures to by-products of pentachlorophenol synthesis, including dioxin-like chemicals.
The objective of this section is to determine what is the role of chemical by-products as potential contributors to reported effects. Although conclusions reached from cancer studies in experimental animals are from different grades of pentachlorophenol and by-products of its synthesis, an analysis of individual components will aid in elucidation of possible mechanisms of carcinogenicity.

A protocol was developed to examine existing murine liver cancer data on different grades of pentachlorophenol and individual by-products. Some of these by-products are dioxins and furans, which have dioxin-like equivalency values (TEQs) that determine biological potencies relative to 2,3,7,8-tetrachlorodibenz-\(p\)-dioxin (TCDD). Details of the protocol are in Appendix D: Approach for the evaluation of by-products of pentachlorophenol synthesis, including dioxin-like chemicals.
Appendix A: Selection of the literature included in the cancer evaluation

This section discusses procedures to identify and select literature relevant for the cancer evaluation, including the literature search strategy and inclusion and exclusion criteria. The relevant literature includes the primary cancer studies in experimental animals, and supporting literature that may be relevant for the interpretation of the studies. The first step in the process is to develop a literature search strategy and associated inclusion/exclusion criteria to identify the relevant database of publications, and the second step is to select the primary studies from this database. Figure 1 is a schematic of the process, which is discussed in detail below.

Figure 1. Selection of literature for cancer evaluation.
1 Identification of relevant citations for cancer evaluation

The identification of the relevant literature includes strategies for searching for citations and inclusion/exclusion questions for selecting the relevant citations from the searches.

Literature searches in three databases, PubMed, Scopus, and Web of Science, are conducted using search terms specific for pentachlorophenol (synonyms, chemical class, metabolites, and exposure scenario) and for the topics covered by the monograph and are outlined in the pentachlorophenol concept document (http://ntp.niehs.nih.gov/go/37803). Specific search terms for the section on Cancer Studies in Experimental Animals are described in Table 1 below.

Table 1. Literature search approach for animal cancer studies with pentachlorophenol

<table>
<thead>
<tr>
<th>Substance</th>
<th>Search terms a</th>
<th>Topic (combined with)a</th>
</tr>
</thead>
<tbody>
<tr>
<td>Penta-chlorophenol synonyms</td>
<td>Pentachlorophenol, 87-86-5 (CASRN), hydroxypentachlorobenzene, pentachlorobenzene, pentachlorophenate, Dowicide EC-7, Dowicide 7</td>
<td>Cancer Studies in Experimental Animals</td>
</tr>
</tbody>
</table>

*aSearch terms have been developed in consultation with an information specialist.

Table 2. Literature search approach for by-products of pentachlorophenol synthesis.

Source

National Toxicology Program (NTP) Technical Reports

- NTP Nomination for Toxicological Evaluation Documents
- NTP RoC Background Documents
- NTP RoC Profiles
- Office of Health and Translation (OHAT, formerly Center for the Evaluation of Risks to Human Reproduction) Monographs
- International Agency for Research on Cancer (IARC) Monographs
- Agency for Toxic Substances and Disease Registry (ATSDR) Toxicological Profiles
- Environmental Protection Agency Integrated Risk Information System (EPA IRIS)
- World Health Organization (Industrial Physical Capability Service) (WHO, IPCS)(a-k below)
  a) Concise International Chemical Assessment Documents
  b) Environmental Health Criteria Monographs
  c) Health and Safety Guides
  d) International Chemical Safety Cards
  e) Joint Expert Committee on Food Additives
  f) Joint Meeting on Pesticide Residues
  g) Keml-Riskline
  h) Pesticide Data Sheets and Documents
  i) Poisons Information Monographs
Based on the reported chemical analyses of pentachlorophenol by-products, a list of potential exposures is generated and the authoritative reports (Table 2) are searched for information on cancer endpoints for these chemicals.

Additional literature searches may be conducted on special topics or issues. The literature strategy will be saved in the databases, which automatically sends out weekly notifications concerning newly identified citations using the saved search strategy.

2 Selection of literature for pentachlorophenol cancer assessment

Citations retrieved from literature searches are uploaded to web-based systematic review software and screened using pre-defined inclusion and exclusion criteria (see below). Multi-level screening of the literature identified from the searches is conducted (see Figure 1); the initial screening is based on titles and abstracts only (Level 1) and will identify relevant studies on pentachlorophenol for sections of the cancer assessment document. In general, the screening of the literature at Level 1 is done using titles and abstracts so the “bar” for excluding literature is very high. Therefore, a more detailed review of the studies for inclusion/exclusion is conducted at Level 2 using the full text article. The Level 1 selections for “Studies of Cancer in Experimental Animals” will include noncancer studies potentially informative for chronic study dose selection or informative for early evidence of preneoplastic or associated non-neoplastic lesions (such as subchronic toxicity studies). Subsequent screening for each section of the document is based on full-text (PDFs) (Level 2). Literature is screened at each level by two reviewers using inclusion/exclusion criteria for each level.

2.1 Selection of studies for inclusion in “Cancer Studies in Experimental Animals” section of monograph (full-text review)

Studies identified for inclusion in the “Studies of Cancer in Experimental Animals” section go through a full text review based on the following inclusion/exclusion questions:

Inclusion/exclusion questions: Level 2 (full text) Animal Tumors

(1) Does this paper contain information that could be informative for cancer assessment in experimental animals?

☑ Yes
☑ (i) this study measures neoplastic (benign, malignant) endpoints.
☑ (ii) this study has non-cancer data that is informative for a cancer assessment, such as reporting preneoplastic lesions
☑ (iii) this study describes non-neoplastic lesions that are considered part of a morphologic continuum to neoplasia.
☑ (iv) this study provides information on chronic study dose selection (such as a subchronic or short-term toxicity study used for chronic study dose selection).
☑ (iv) Other
(2) If the answer to Question #1 is "No," select the reason below for excluding it from review.

☐ (i) It does not contain relevant information on the candidate substance.
☐ (ii) It is related to the candidate substance (or one of its metabolites, analogues, or chemical class), but the paper does not contain relevant information for an assessment of animal tumors.
☐ (iii) Other.
Appendix B: Assessment of the quality of the individual animal cancer studies

Each of these primary studies will be systematically evaluated in a two-step process. First studies will be evaluated for whether the level of detail reported for key elements of study design, experimental procedures, and cancer endpoints were adequate for evaluating its quality and interpreting its results. Second, studies meeting these reporting criteria will be assessed for concerns of study quality that might negatively impact the ability to evaluate carcinogenicity.

Study quality assessment

Study quality will be assessed using questions related to the following study performance elements: substance characterization, animal husbandry, study design, endpoint assessment, and data interpretation. In most cases, each question inquires whether there are concerns (minimal, some, major, or no information reported) that the quality of a specific study element is adequate for attributing any neoplastic endpoints to exposure to pentachlorophenol and byproducts of its production. In general, the ranking of the concerns for the study elements is based on how far each study element deviates from the ideal (see below).

The assessment of the overall quality of a study is based on consideration of the individual elements and how they impact the usefulness of that study in assessing carcinogenicity. Studies that were given the most weight in the evaluation (e.g., those with no or minimal concerns in key elements) are those with the following key characteristics:

- Uses a chemical preparation that is representative of the candidate substance (in terms of purity and stability) so that any observed effects can be attributed to the candidate substance.
- Has no evidence of poor animal husbandry conditions (such as high mortality due to infection). Often information on animal husbandry conditions is not known and while this information is desirable, it is not a requirement.
- Exposes animals to high enough doses (resulting in tolerable toxicities) for a sufficiently long duration (approaching the lifetime of the animal), but not to a dose that limits survival over the exposure period. The use of more than one dose level is ideal, but is not a requirement.
- Has an appropriate comparison group (e.g., ideally unexposed, sham-treated concurrent controls). The absence of an appropriate control group, by itself, is sufficient for judging a study to be inadequate for cancer evaluation.
- Has adequate statistical power to detect an effect, which is based on the number of animals used in a study, the incidence of tumors in control vs. treated group, and the rarity of the tumor.
- Performs full necropsies and histopathological examinations on all tissues. Ideally, animals are exposed to multiple doses that allow for statistical comparisons with the control group and dose-response analysis.

An ideal study would have the following characteristics, which are related to interpreting the study. In general, with the exception of route of exposure, these do not contribute as much weight to the overall evaluation of the study as the characteristics related to the validity of the study discussed above.

- The use of an exposure route comparable to human exposure.
• The use of animal model that is sensitive for detecting tumors and does not have high background rates for the observed tumors. Studies in both sexes are more informative than those testing only one sex. Often this information is not available.
• Availability of historical control data, which can be helpful in assessing the significance of a finding, especially in the case of rare tumors, lower powered studies, or assessment of background tumor incidences. Rare tumors will be considered in the assessment even if their incidences do not reach significance.
• Appropriate reporting of incidence data and statistical methods. If statistical tests are not reported, the study should at a minimum present incidence data for specific tumors so that statistical tests can be run.

Studies having elements that are judged to have some or major concerns may still be considered in the evaluation or can be considered to provide support to the more informative studies. It should also be noted that some concerns about a study element (such as inadequate observation and exposure period and statistical power) would decrease the sensitivity of a study to detect an effect; however, if despite these limitations positive findings were described, these studies would inform a cancer assessment.

Questions for the assessment of the quality of cancer studies in experimental animals are as follows:

1 **Substance characterization**
Are there concerns that the purity solubility and stability of the chemical are not adequate for attributing any neoplastic effects to the substance?

2 **Animal husbandry**
Are there concerns that the quality of the animal husbandry (e.g., care, diet, maintenance, and disease surveillance) is not adequate for attributing any neoplastic effects to the substance?

3 **Study design**
Are there concerns that the study design did not include randomization of animals to dose groups and blinding of dose groups?
Are there concerns that the dosing regimen (dose selection and dose groups, or other factors) is either not adequate for detecting a neoplastic effect (if present) or for attributing any tumor effects to the substance?
Are there concerns that the study duration (exposure and observation) is not adequate to detect a neoplastic effect, if present?
Are there concerns that the current control group was not adequate for evaluating the study?
Are there concerns that the study does not have adequate statistical power (number of animal per exposure and control group) to detect a neoplastic effect, if present?

4 **Clinical observations, necropsy and pathology (Endpoint assessment)**
Are there concerns that the assessment of study outcome (gross and microscopic tissue analysis) was not done blind?
Are there concerns that the methods to access tumor outcome and the pathology procedures (necropsy, histology, or diagnosis) are not adequate for attributing the effects to the exposure?

5 Data interpretation

Are there concerns that survival-related effects could affect attributing the study findings to the exposure?

Are there concerns that the route of exposure is not adequate for evaluating the potential for human carcinogenicity?

Are there concerns about the animal model (source, species, strain, or sex) that could affect study interpretation?

Are historical control data reported? If not, this would be a concern for rare tumors, or for tumors with high background.

Are there concerns that reporting of the data and statistical analysis are inadequate for evaluating the results?

6 Overall assessment of study quality and utility for cancer assessment

Does this study have utility for cancer assessment? What is the overall level of concern for the quality of the study, and how would any concerns affect its interpretation?
Appendix C: Assessment of the level of evidence of carcinogenicity from studies in experimental animals.

This section outlines the approaches for synthesizing the findings across the body of studies for each cancer endpoint and for making a recommendation on the level of evidence (e.g., sufficient or inadequate) of the carcinogenicity of pentachlorophenol and by-products of its synthesis from studies in experimental animals. The most informative studies to detect an effect will be identified by using the guidelines and checklist described in Appendix C, and these studies are given the most weight in the assessment. The following factors are taken into consideration in determining whether an effect is treatment related: statistical significance with respect to concurrent controls and dose-related trends, non-neoplastic lesions, lesion progression, decreased latency, tumor multiplicity and survival, historical control range, animal species and strain, and rarity of tumor.

The application of the RoC listing criteria to the body of studies on pentachlorophenol includes evaluating whether there is an increased incidence of malignant and/or a combination of malignant and benign tumors (1) in multiple species or at multiple tissue sites, or (2) by multiple routes of exposure, or (3) to an unusual degree with regard to incidence, site, or age at onset.
Appendix D: Approach for the evaluation of by-products of pentachlorophenol synthesis, including dioxin-like chemicals

1 Identification of all chemical by-products and contribution to carcinogenic potency.

The evaluation of a potential association with carcinogenicity will take into account the following:

- Identification of pentachlorophenol by-products in preparations (such as technical, commercial, and purified grades of pentachlorophenol) and evidence related to their carcinogenic potency.
- Assessment of the level of exposure to the by-product compared with exposure to pentachlorophenol.
- Determination of whether there are peer-reviewed cancer studies (or toxicity studies) on the by-products (or on the chemical class if cancer studies on specific chemicals are not available); note doses, species and strain, duration and neoplastic endpoints. Authoritative sources (see Appendix A, Table 2) will be used for identification of these endpoints.

2 Identification of dioxin-like chemical by-products and relative contribution of their dioxin-like properties to carcinogenic potency.

A number of potential chemicals with dioxin-like (2,3,7,8-TCDD) activity have been reported as by-products formed in the manufacture of pentachlorophenol. For these chemicals, the relative contribution of their dioxin-like properties can be estimated based on dioxin toxic equivalent factors (TEQs), which can be calculated from the toxic equivalency factor (TEF) for individual dioxin-like chemicals times the concentration of the chemical in the mixture. Total dioxin-like equivalency for the mixture is the sum of individual TEQs. With this method, it is assumed that the dioxin-like activity of individual chemicals is additive in a mixture.

- Calculate TEQ value for dioxins, furans, etc., having toxic equivalent factors (TEF) reported, using the highest dose administered of pentachlorophenol; err on the side of worst case scenario and note any assumptions (dose, congener, use level of detection value if reported below level of detection).
- Sum TEQs for dioxin-like by-products for a given dose group.
- Note assumptions in text or in footnote.
- Translate into relative amount of dioxin equivalents delivered to animal by summing TEQs for each dioxin-like by-product.

---

• Compare summed TEQ to known cancer exposure for 2,3,7,8-TCDD from a similar experimental animal study.

• Determine relative contribution of TEQs to neoplastic outcome. Compare neoplastic outcome (liver cancer in mice) for TCDD with that for pentachlorophenol exposure in a comparable study.

• Note all assumptions with respect to route, chemical composition, level of chemical detection.