Protocol for Cancer Assessment of Animal Studies for the Report on Carcinogens Monograph on PCP

Background Information

Pentachlorophenol (PCP, 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. People have been and are currently exposed to PCP from its production and use in lumber treatment, and in other lumber-related occupations, and they were exposed in the past from its broad use as a pesticide. PCP's use in the United States was restricted to wood preservation in 1984, and it can no longer be used on wood in residential or agricultural buildings. Use in the United States is restricted primarily to the treatment of utility poles and cross arms, and PCP is also used in the treatment of railroad ties and wharf pilings. Current use in Canada includes bridge decking, fence posts, exterior laminated timbers, piles, and wood poles. Although its use as a wood-preservative has been limited to non-residential and non-agricultural applications since 1984, there is still potential for occupational and environmental exposure in the United States.[1] It was detected in ambient air of a community of residents near a wood treatment facility, in indoor air, in food, and in the urine from children and adults.[2] PCP and its sodium salt have been selected as candidate substances 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 PCP that are adequate for evaluating its potential carcinogenicity.

The proposed approach for conducting a cancer evaluation of PCP – including the literature search strategy, the scope and focus of the monograph, and the approaches for obtaining scientific and public input to address the key scientific questions and issues – is available at http://ntp.niehs.nih.gov/go/37803 under "Report on Carcinogens (RoC) Concepts." This protocol is limited to the procedures used to prepare the animal cancer studies section of the draft RoC monograph and 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.

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[1] ATSDR. 2001. *Toxicological Profile for Pentachlorophenol. September 2001.* *http://www.atsdr.cdc.gov/toxprofiles/tp51.pdf.* Accessed 4/2012.

[2] Wilson NK, Chaung JC, Morgan MK, Lordo RA, Sheldon LS. 2007. An observational study of the potential exposures of preschool children to pentachlorophenol, bisphenol-A, and nonylphenol at home and daycare. *Environ Res* 103(1):9-20.

Key Scientific Questions

A primary question for the evaluation is:

- What is the level of evidence (sufficient or not sufficient) of carcinogenicity of PCP from animal studies?

Secondary questions are as follows:

- What are the methodological strengths and limitations of the studies?
- What are the tissue sites?
- Can exposure to contaminants be ruled out as potential contributors to reported effects?

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 E.

Appendix A: Selection of the literature included in the evaluation of cancer studies in experimental animals (Table A-1). Cancer studies on chemical contaminants in PCP will be from secondary sources (Table A-2).

Appendix B: Systematic extraction of data from the cancer studies in experimental animals.

Appendix C: Assessment of the quality of the individual animal cancer studies.

Appendix D: Approach for the evaluation of co-exposures to impurities in PCP and assessment of dioxin-like contaminants.

Appendix E: Assessment of the level of evidence of carcinogenicity (sufficient or inadequate) of PCP from studies in experimental animals.

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 relavant 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.

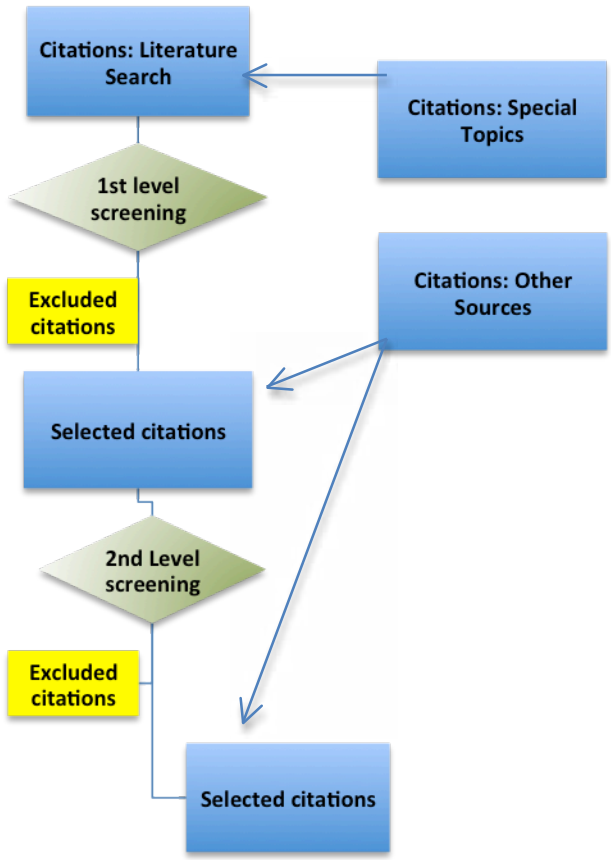

Figure 1. Selection of literature for cancer evaluation.

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 PCP 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 PCP.

Substance	Search terms [a]	Topic (combined with)[a]
Penta-chlorophenol synonyms	Pentachlorophenol, 87-86-5 (CASRN), hydroxypentachlorobenzene, pentachlorobenzene, pentachlorophenate, Dowicide EC-7, Dowicide 7	Cancer Studies in Experimental Animals

[a]Search terms have been developed in consultation with an information specialist.

Table 2. Literature search approach for co-exposures to impurities in PCP.

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) KemI-Risline
    h) Pesticide Data Sheets and Documents
    i) Poisons Information Monographs
    j) Screening Information Data Set for High Production Volume Chemicals
    k) UK Poison Information Documents
- California EPA Prop 65 Hazard Identification Documents

Based on the reported chemical analyses of impurities in PCP, a list of potential co-exposures is generated and 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 PCP 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 PCP 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 of 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*

- ❑ *No*

(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. Systematic extraction of data from cancer studies in experimental animals.

Two independent reviewers will extract data (such as methods and findings) from the individual studies into a database in a systematic manner using standardized instructions and questions. Data entry will be checked by the reviewers and any discrepant entries will be resolved between the reviewers by referring to the original data source and discussion. The database contains "fields" that are specific for the different types of extracted information. The fields will be used to populate tables used in the monograph. The assessment of study quality (see Appendix C) will also be entered into the database.

Appendix C: Assessment of the quality of the individual animal cancer studies

Each primary study will be systematically evaluated to determine if it is informative for a cancer assessment. Studies that will be given the most weight in the evaluation are those that are of a sufficiently long duration to identify a cancer endpoint, (ideally an exposure approaching the lifetime of the animal), provide a detailed account of the study design and data collection, and use dose levels that include or approach the maximum tolerated dose. Ideally, studies should use an exposure route comparable to human exposure and appropriate statistical methods in reporting of results. Comparison with historical control values is sometimes helpful in assessing the significance of a finding, especially in the case of rare tumors, lower powered studies or assessment of background tumor incidences. The number of animals used in a study, the incidence of tumors in control vs. treated group, and the rarity of a tumor influence the statistical power of a study to detect an effect and are parameters that need to be taken into account in study design and results assessment. *Post hoc* power calculations can be performed. However, rare tumors will be considered in the assessment even if their incidence does not reach significance. Study performance elements for evaluating the different components of study quality are described below.

1 Substance characterization

- Is the chemistry of the substance well characterized?
- Are the purity, solubility, and stability of the substance adequate for attributing any adverse effects to the substance?

2 Animal husbandry

- Are the source, species, and strain of the animals adequately described?
- Are the care, diet, housing and maintenance of the animals adequate for attributing any adverse effects to the substance? Is there any evidence that infection, diet or other factors could have played a role in causing any adverse effects?
- Were control animals housed in the same room, and tested at the same time under the same conditions as the dosed groups?

3 Study design

- Animal model: Are the species and sex appropriate for determination of any adverse effect?
- Dosing and observation conditions: Are the study period, dosing period, route of exposure, and doses used adequate for determination of any adverse effect?
- Statistical Power: Does the study have adequate statistical power to detect an adverse effect if present?

4 Clinical observations, necropsy and pathology

- Was a full necropsy done on these animals and was histopathology done on tissues from all organs?
- Are pathology procedures well described and adequate for determination for any adverse effect?

5 Data reporting and statistical methods

- Is data reporting well characterized?

- Have tumors (benign/malignant) from the same organ been combined? If so, do they originate from the same cell type? *e.g.*- fibrosarcoma would not be combined with adenoma.
- Are the statistical methods adequate to attribute an adverse effect?
- Are appropriate historical control data available?

6 Overall, is this study informative for cancer assessment and why or why not?

Appendix D: Approach for the evaluation of co-exposures to impurities in PCP, including dioxin-like contaminants

NTP will convene an information group[3] to discuss the potential effects of contaminants of PCP to overall carcinogenic potency in experimental animals. Approximately 2 to 4 scientists with substance-specific expertise will independently review animal data, discuss, and provide input to the Office of the Report on Carcinogens.

1 Identification of all chemical contaminants and contribution to carcinogenic potency.

A key question in the evaluation of the level of evidence from animal cancer studies is whether an association (if any) between exposure to PCP and cancer can be explained by the presence of impurities in the PCP preparation. The evaluation of a potential association will take into account the following:

- Identification of impurities in PCP preparations (such as technical, commercial, and purified grades of PCP) and evidence related to their carcinogenic potency.
- Assessment of the level of exposure to the impurity compared with exposure to PCP.
- Determination of whether there are peer-reviewed cancer studies (or toxicity studies) on the contaminants (or on the chemical class if cancer studies on specific chemicals are not available); note no observed effect levels (NOELs), 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 contaminants 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 impurities formed in the manufacture of PCP. For these chemicals, the relative contribution of their dioxin-like properties can be estimated based on dioxin toxic equivalent factors (TEQs).

- Calculate TEQ value for dioxins, furans, *et cetera*, having toxic equivalent factor (TEF) reported[4] using the highest dose administered of PCP; 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 contaminants for PCP for a given dose group.
- Note assumptions in text or in footnote.

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[3] An information group is a group assembled for the purpose of exchanging facts or information and is not covered by the Federal Advisory Committee Act. Members provide input on an individual basis and not from the group as a whole.

[4] M. Van den Berg, L.S. Birnbaum, M. Denison, M. De Vito, W. Farland, M. Feeley, H. Fiedler, H. Hakansson, A. Hanberg, L. Haws, M. Rose, S. Safe, D. Schrenk, C. Tohyama, A. Tritscher, J. Tuomisto, M. Tysklind, N. Walker, R.E. Peterson, The 2005 World Health Organization reevaluation of human and mammalian toxic equivalency factors for dioxins and dioxin-like compounds, Toxicol. Sci. 93 (2006) 223–241.

- Translate into relative amount of dioxin equivalents delivered to animal by summing TEQs for each contaminant.
- Compare summed TEQ to known cancer NOEL for 2,3,7,8 TCDD.
- Determine relative contribution of TEQs to neoplastic outcome and compare the target cancer sites for TCDD with those for PCP exposure.

Appendix E. 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 PCP 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 PCP 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.