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Comparison of carcinogenic potency across life stages: implications for the assessment of transplacental cancer risk

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Abstract

Cancer development from *in utero* exposure has been documented for a variety of agents with the most commonly studied compounds exhibiting mutagenic and genotoxic carcinogen properties. Age-dependent adjustment factors (ADAFs) are applied by the US Environmental Protection Agency to many such carcinogens when assessing cancer risk from early postnatal (PN) exposures; however, this approach has not been widely considered for transplacental (TP) exposure. To explore this question and further evaluate prenatal susceptibility a database of early life animal studies developed by California Office of Environmental Health Hazard Assessment (OEHHA) enhanced with additional literature searching was evaluated. Nine genotoxic carcinogens and one mixture (cigarette smoke) have data available via TP only, PN only and adult-only protocols. Potency comparisons across these lifestages displayed similarly greater potency in male liver and brain compared to adult-only exposure. Both TP and PN exposures were not markedly different than adult-only for other targets such as female liver and blood-borne tumors. Similarity in TP and PN targets and potency for carcinogen action suggests that a 10-fold ADAF may be applied for TP exposure as is currently applied to PN exposure. A similar conclusion was reached by OEHHA. The implications of this heightened TP vulnerability are greatest for less-than-lifetime exposure and this approach might be used to assess the level of cancer risk from exposures during pregnancy. A case example employing a mutagenic flame retardant was used to exemplify application of a TP ADAF for evaluating risks during pregnancy.

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Keywords

Transplacental; in utero; pregnancy; cancer; carcinogen

Introduction

A variety of agents were reported to exert a carcinogenic effect from gestational exposure thus demonstrating that the carcinogen and/or its active metabolites traverse the placenta (Autrup 1993; Miller 2004). Both genotoxic carcinogens such as nitrosamines and polycyclic aromatic hydrocarbons (PAHs) and agents considered to act via non-genotoxic mechanisms including arsenic or diethylstilbestrol exhibited transplacental (TP) activity, although the majority of available data was generated with genotoxicants. Another window of carcinogen vulnerability is the early postnatal (PN) period with this evidence leading to the development of age-dependent-adjustment to the development of age-dependent-adjustment factors (ADAFs) by the US Environmental Protection Agency (Barton et al. 2005; USEPA 2005). Compared to adults, USEPA derived an estimate of 10-fold greater potency for exposure during the first 2 years of life, and a threefold higher potency for exposure between ages 3 and 15 years. These ADAFs pertain to carcinogens acting via a mutagenic mode of action (MOA). These potency adjustments provide a means to incorporate postnatal carcinogen vulnerability into calculations of lifetime and less-than-lifetime cancer risk.

In contrast to PN exposure, TP exposure in most cases has not been factored into cancer risk assessment. The only compilation of TP carcinogenesis data from animal studies is a review conducted by the California Office of Environmental Health Hazard Assessment (OEHHA) 2009a. This review was part of a larger evaluation of early life carcinogenic evidence that covered both TP and PN data, encompassing 23 chemicals across 37 studies (OEHHA) 2009a. OEHHA developed lifestage potency (LP) ratios for TP/adult-only exposure for 22 datasets involving 14 chemicals in which there are 12 cases of TP exposure exerting greater effect than adult-only exposure, in some cases by nearly 100fold. The OEHHA analysis also found greater LP ratios from PN as opposed to adult-only exposure with OEHHA analysis compatible with PN ADAF established by USEPA of 10-fold. The OEHHA 2009 (a) report forms the starting point of the current analysis, and the majority of the carcinogens evaluated by OEHHA were included in the USEPA (2005) analysis as part of the evidence base for the ADAF approach for carcinogens acting via a mutagenic MOA. Genotoxicant is a generalized term that includes agents acting via a mutagenic MOA, and used here to describe chemicals or groups of chemicals that have not been formally reviewed within the context of the USEPA Supplemental Cancer Guidance (U.S. Environmental Protection Agency (U.S. EPA) 2005).

Given that ADAFs are applied to PN exposure, a key question is whether these or some other methods should be applied to TP exposure and at which level. One way to assess this is by comparing TP and PN cancer results from animal testing. If these life stages are similarly vulnerable then perhaps the 10-fold ADAF also needs to be applied to TP exposures. The OEHHA (2009a) analysis shows a somewhat greater vulnerability from PN

compared to TP exposure (mean lifestage potency (LP) ratios to adult of 27.08 and 7.03, respectively), but those ratios were not necessarily for the same chemicals and target organs. Further, the OEHHHA analysis demonstrated a great deal of variability in LP results across chemicals. The current analysis further evaluates the chemicals reported by OEHHHA as having sufficient animal data to calculate TP, PN, and adult potency and compares these potency estimates across life stages for case study chemicals. Note that the term “transplacental” is meant to indicate carcinogenesis due to chemicals crossing the placenta and damaging fetal tissues, either as parent or metabolite, as evidenced for a wide range of carcinogens (Botsivali and Kyrtopoulos 2019), rather than as a result of effects on the maternal system or the placenta itself.

The emphasis on TP carcinogenesis is reinforced by evidence in humans. A variety of agents have been associated with the development of cancer in offspring from maternal exposures, with the most studied example being diethylstilbestrol (DES) given as a pharmacologic estrogen during pregnancy to prevent miscarriage and preterm labor. Epidemiologic investigation showed a 40-fold increase in reproductive tract tumors in DES daughters, while the effect in DES-exposed mothers was not nearly as dramatic, primarily a rise in breast cancer risk (Titus-Ernstoff et al. 2001; Troisi et al. 2007). Another endocrine active agent, dichlorodiphenyltrichloroethane (DDT) was associated with human breast cancer if exposure occurred *in utero* or in young children prior to age 14 (Cohn et al. 2007, 2015).

While these examples highlight non-genotoxic and potential hormonal mechanisms in human TP carcinogenesis, there is evidence for genotoxicants such as nitroso compounds as well as radiation exposure (Brent 2014; IARC 2010). Regarding nitroso compounds, maternal dietary nitrate and nitrosating pharmaceuticals were correlated with brain cancer in offspring (Bunin et al. 1994; McKean-Cowdin et al. 2003; Preston-Martin 1989). A genetic polymorphism in glutathione transferase that renders this nitroso defense mechanism less active appears to increase prenatal vulnerability to this source of dietary carcinogen (Neilsen et al. 2011). Thus, human evidence is consistent with findings in animals of brain tumors from TP or PN nitroso exposure (Naito, Naito, and Ito 1981; Rice 1981; Zeller, Ivankovic, and Zeller 1978).

The epidemiological and animal evidence for TP carcinogenesis make this a key lifestage for incorporation into human health risk assessment (NRC 2009). However, the empirical evidence has thus far not been analyzed in a manner that is comparable to the USEPA PN approach for use in risk assessment (EPA, 2005). Thus, the objectives of the current review and analysis were to:

1. Develop a framework for the review of animal early life carcinogenesis data in a systematic manner that facilitates tiering of studies with respect to utility for quantitative evaluation of potency across life stages (TP, PN, adult).
2. Apply this tiering framework to studies reporting TP, PN, and adult-only carcinogenesis for data-rich case study chemicals for which both the TP/adult and PN/adult potency comparison is possible according to the OEHHHA (2009a) review.

3. Compare sex and target organ-specific carcinogenic potency between TP and PN studies.
4. Update the OEHHHA (2009a) review with additional literature search and evaluation.
5. Consider methods for applying TP cancer data to risk assessment to assess lifetime, as well as short term, cancer risk related to exposure during pregnancy.

Methods

The OEHHHA (2009a) report provided the starting dataset, and additional details were obtained from OEHHHA regarding the manner in which information from these studies was applied to calculate cancer LP ratios. Data were reviewed to identify chemicals which had cancer results for all three life stages of interest: TP, PN and adult-only exposure periods. All studies providing relevant data as identified in the OEHHHA report were retrieved. The accumulated information was entered into a database which detailed key study design features (sex, dose route, day(s) of dosing, dose rate, cumulative doses) and study results (tumor incidence, target site). This database is presented in the Supplemental Tables associated with this manuscript. LP ratios were calculated by OEHHHA for the early life period relative to adult-only exposure. Most studies only reported a single dose level and so the LP was estimated as the tumor response divided by the cumulative dose for each life stage, sex, and target organ compared. Potency calculations presented in this manuscript do not include the time-of-dosing factor introduced by OEHHHA to account for the greater opportunity for latent effects to occur after early-life exposure than after adult exposure. Since the time-of-dosing factor is similar for the prenatal and postnatal periods it has only a minor effect on the comparison between these exposure periods.

The database of early life and adult-only studies was evaluated according to a tiering framework as described below. A major criterion for inclusion of a study by OEHHHA in its dataset was for multiple life stages (i.e., at least two of the following life stages: TP, PN, juvenile and adult) to be present within the same study. This and other investigation features which make a study or a group of studies optimal for comparing potency across life stages was compiled into the tiering framework, as described below, in an effort to systematize study comparability. The framework was informed by both the OEHHHA (2009a) (TP and PN) and U.S. Environmental Protection Agency (U.S. EPA) (2005) (PN only) criteria for study inclusion in their respective databases.

Tiering framework

The tiering framework focuses upon study design, reporting, and other quality issues as many TP and PN animal experiments were conducted in a screening manner that had a number of common limitations. These include incomplete histopathology, small number of animals, single-dose level, and short observation periods (i.e., less than 1 year) over which tumors might develop. While these factors tend to make it more difficult to detect a carcinogenic effect and evaluate dose response, if the early life and adult-only protocols are similar in design, the study may still provide useful early life/adult comparisons. Factors such as whether the different life stages had a similar protocol such as species, strain, dose

route or observation period, as well as general study reporting and quality objectives, were considered in this tiering evaluation.

Recognition of these limitations and review of inclusion criteria used by OEHHA (2009a) and U.S. Environmental Protection Agency (U.S. EPA) (2005) led to the identification of the following 13 attributes specific to early life carcinogenesis studies (Table 1). The study attributes are used to categorize investigations and ultimately the database for a particular chemical into evidence tiers. Core attributes need to be present as they are integral to study quality and the ability to compare carcinogenic effects across life stages. One might think of these as “minimal inclusion criteria”. *Key* attributes further improve the early life database but studies are not invalidated if one or more key attributes are missing. *Desirable* attributes increase the amount of data a study provides and enhances the sensitivity of a design. These would normally be written into a cancer bioassay protocol but given the variability and screening nature of much of the published literature in this area, such aspects are often missing.

The attributes presented in Table 1 are used to segregate studies and groupings of investigations for a single chemical into evidence tiers as follows:

- Tier 1: most useful for quantitative comparison of potency across life stages, having all core attributes and most key attributes. Studies may be lacking one key attribute that affects power of the test to detect an effect (e.g., shorter observation period, smaller number on test, only one dose level) but could still be considered Tier 1 if the dose rate on every day of dosing is known (Attribute VI) and the dose route (Attribute VII) and species/strain (Attribute VIII) are the same across life stages tested. The estimate of potency for bioassays that were run in parallel at the same lab is most directly comparable across life stages.
- Tier 2: all core attributes are present but several (2–3) key attributes which might affect the comparability of potency across life stages are at issue (e.g., different species/strain, Attribute IX; dose route). These may still form the basis for quantitative dose-response comparisons, albeit with lower confidence. In terms of Key attributes, Tier 2 studies would at a minimum contain Attribute VI, administered dose.
- Tier 3: all core attributes are present, but these studies are the least useful for quantitative comparisons. One common reason is that Attribute VI is deficient due to lactational exposure in the PN study as the dose to the nursing pup is not known, only the dose to the mother. How the maternal dose converts to a pup dose cannot be estimated in most cases because breast milk concentrations of chemical are typically not measured in rodent toxicology studies. Another reason for the Tier 3 categorization is when the dose response is not informative, e.g., when the maximal rate of tumor formation is reached in the low dose group (or only dose group) such that a range for a graded dose response was superseded. This makes potency calculations more uncertain.

Calculation of a composite TP/PN ratio across numerous case study chemicals is conducted by weighting the individual ratios according to evidence tier. Chemicals with a Tier 1 level

of evidence for the potency comparison are weighted at full strength (weighting factor = 1) while Tier 2 is weighted at half strength (factor = 0.5) and Tier 3 at quarter strength (factor = 0.25). These tiers of evidence are brought together into an overall-weighted TP/PN ratio involving case study chemicals (e.g., chemicals a, b and c) as follows:

$$\text{Weight Study}_a = \text{Tier}_a / (\text{Tier}_a + \text{Tier}_b + \text{Tier}_c)$$

Where: Weight Study_a is the weighting factor assigned to case study example “a” (Case Study_a) according to its tier ranking, likewise for Case Study_b and Case Study_c. For example, if there are 3 study chemicals for a particular target organ, one at Tier 1, one at Tier 2 and one at Tier 3, the above equations yield a 57% weight for Case Study_a (the Tier 1 case), 29% for Case Study_b (the Tier 2 case) and 14% for Case Study_c (the Tier 3 case).

Tier_a is defined by the evidence based weighting factors of 1, 0.5 or 0.25 as described above.

$$\text{Weighted TP/PN} = (\text{TP/PN})_a * \text{Weight Study}_a + (\text{TP/PN})_b * \text{Weight Study}_b + (\text{TP/PN})_c * \text{Weight Study}_c$$

Where:

(TP/PN)_a is transplacental to postnatal potency ratio for Case Study_a;

This tier weight-averaging approach was applied to all endpoints with a heightened response in early life, either TP, PN or both.

A literature search to identify additional compounds tested by TP, PN and older ages (adult only or at least juveniles beginning at 1 month of age) exposures was conducted through the end of February 2019 with search terms and results as described in Supplement Table 10.

Results

Review of the OEHHA (2009a) report identified nine carcinogens which have animal tumor data for both transplacental (TP) and postnatal (PN) exposure and whose results may be compared to older juvenile/adult-only studies: benzidine, butylnitrosourea (BNU), diethylnitrosamine (DEN), ethylnitrosobiuret (ENB), ethylnitrosourea (ENU), 3-methylcholanthrene (3-MC), safrole, urethane and vinyl chloride (VC). All of these carcinogens are also genotoxicants. The California OEHHA cancer potency was based upon multiple tumor sites in the same experiment, and did not provide results for specific target organs and thus was not possible to make a direct comparison across lifestage for matching cancer sites. Review of the original studies for the nine chemicals in the OEHHA database which have TP and PN data indicated that separate comparisons are possible for five target organs: liver, brain, kidney, lung and leukemia/lymphoma. The tiering framework was used to assign levels of evidence to each study and life stage comparison for each of these cancer targets. Table 2 provides results of the evidence tiering process for the nine chemicals while the database for each chemical is provided in Supplement Tables 1–9.

Of the 13 studies useful for across life stage comparisons of cancer potency for these nine chemicals, only VC evidence (Maltoni et al. 1981) is considered the highest tier of evidence

(Tier 1). In that case, the species, strain, and dose route were invariant across life stage with sufficient information reported to be able to estimate dose rate on each day of dosing. Further, the liver tumor response was substantial but not maximal at the dose levels selected so that while still limited, there is some indication of dose response. Although the databases for the other eight carcinogens in this analysis have limitations which place them into Tiers 2 or 3 (Table 2), they still provide an indication of relative potency across life stages. Because of the few chemicals identified in OEHHA (2009a) with TP, PN and adult data, the current analysis joins all three tiers in a weighted analysis of TP to PN potency comparison as described in Methods.

Tumor potency organized by target organ is presented in Figure 1–6. Each figure shows the ratio of early life stages to adult (TP/adult and PN/adult) and then also provides the TP/PN ratio for each chemical, cancer target and sex (where available). Supplementary tables illustrate the tumor bioassay results that were used to create the ratios depicted in the figures. The TP and PN response across five different chemicals involving three different tiers of evidence is generally similar in livers of male mice and rats (Figure 1). As an example, the potency in both the TP and PN periods was markedly greater than adult-only exposure for 3-methylcholanthrene, urethane (mice and rats separately analyzed), safrole and benzidine. The TP/PN potency ratio was greater than one in the case of urethane-induced liver tumors but less than one in all other comparisons (Figure 1). The case where the TP and PN results were most dissimilar was VC-induced tumors in male liver. The TP response is only numerically above adult exposure while the PN response was markedly elevated approximately 30 fold above adult or TP potency (Figure 1).

In contrast to male liver, female liver was less responsive to carcinogenesis in the three cases where early life to adult comparisons was possible (Figure 2). For safrole and benzidine, both the TP and PN liver tumor responses were low and similar to adults, whereas the liver tumor response was clearly elevated by early-life exposure in males (Figure 1). While the 3-MC liver tumor response was also muted in females, the PN response remained elevated, creating a low TP/PN ratio for this agent in female liver (Figure 2).

The limited comparisons possible for brain suggest that TP and PN potencies are similarly low for BNU and similarly high for ENB and ENU with the latter two chemicals displaying a potent brain cancer response from early life exposure (Figure 3). The TP/PN ratio was near unity for all three chemicals with the ratio numerically in favor of greater TP sensitivity in two cases and greater PN sensitivity in one. Regarding the other organ-specific comparisons, kidney (Figure 4), lung (Figure 5) and lymphoma (Figure 6, less data are available from these nine case-study chemicals to compare potency across the three life stages (TP, PN, adult)). In the case of kidney, both a Tier 1 (VC) and Tier 2 (BNU) chemical example demonstrated little responsiveness from postnatal or adult exposure but both showed significant consequences resulting from TP exposure (Figure 4). The rate of kidney tumor formation was relatively low in both cases (10% TP BNU, 6% TP VC) but background rate of kidney cancer was low in these rats. For VC, the elevated kidney cancer response is in contrast to that for liver cancer which was not affected by TP exposure in the same experiment.

Comparison of lung tumor response across life stages showed no marked change in early life relative to adult-only exposure (Figure 5) which was mainly due to the high rate of lung tumors in adults in these examples (83% urethane, 37% to 80% DEN). Since the TP and PN lung tumor response was marked in these investigations, there was no significant alteration relative to adults and the design may not have been optimal to view an effect. As described below, a recent bioassay involving cigarette smoke exposure noted an elevated lung tumor response in early life relative to a low rate in the adult-only exposure group. Figure 6 shows the results for blood-borne cancers, in which there are two examples of Tier 2 levels of evidence. BNU treatment during TP or PN development did not induce leukemia while the adult-only treatment produced carcinoma (18%). In the case of 3-MC administration in mice, both TP and PN administration was associated with increased lymphoma incidence (42–43%) but this was less than the adult lymphoma rate (58%) from the same dosing regimen. These findings are summarized in Figure 6 indicate that both early life stages did not markedly affect the occurrence of leukemia/lymphoma relative to adult-only exposure in these bioassays.

While the primary focus of this effort was to analyze the case examples from OEHHHA (2009a) in which the potency during TP and PN life stages could be compared, an updated literature search and evaluation (Supplement Table 10) identified a high quality, Tier 1 evaluation of the lung adenoma+carcinoma response to cigarette smoke in Swiss mice exposed during the entire gestational period (19 days) in comparison to the entire neonatal/juvenile period (120 days) vs adult-only exposure (Balansky et al. 2012). This investigation constitutes Tier 1 evidence because of uniform exposure protocols encompassing the entire early life stages of interest against a comparable period of exposure and latency evaluation in an adult-only exposure group. Figure 7 shows a greater tumor potency from both TP and PN exposure relative to adult, the latter exhibiting no change in lung cancer from 4 months of daily exposure. The TP and PN lung adenoma+carcinoma responses were similar but the potency calculation was greater for TP because the exposure was for only 19 days compared to PN, which was for 120 days. This calculation may overstate the difference between TP and PN vulnerability as there may be a shorter window of PN vulnerability than the first 4 months of life but that is the only exposure window that was tested for PN exposure. The shorter the dosing period the lower the cumulative dose and thus the greater is the potency estimate for a given tumor response. In addition, the staging and multiplicity of lung tumor responses indicated greater effect in the PN period as follows: 10% carcinomas in PN vs 0% TP (Balansky et al. 2012) which is not surprising given the direct exposure of smoke to the contact site in PN animals as opposed to only indirect exposure via the dam in TP animals.

Table 3 provides a tiering-based weighted potency comparison between TP and PN life stages for the chemical examples and target organs presented above. The strength of evidence in terms of Tier 1, 2 or 3 for each study is illustrated in Figures 1–7. Liver is divided by sex because this target has the greatest amount of data for a male/female distinction in early life. Table 3 excludes leukemia/lymphoma since either TP or PN exposures failed to markedly affect the response relative to adult-only exposure.

The composite weighted TP/PN ratios shown in Table 3 suggest a similar potency across these early life stages for these various target organs as the composite ratios were near unity

in four of the five cases. The higher composite ratio for kidney raises the possibility that kidney may be at enhanced vulnerability during the TP period relative to PN and adult, but this is only based upon two studies. Liver (males) and brain are at clearly heightened response in both TP and PN periods with the difference between these periods apparently not dramatic, showing composite TP/PN ratios close to one. However, there might be exceptions as VC and 3-MC were significantly more potent in the PN than TP period. Lung was not altered from early life exposure in two of the case studies (urethane, DEN), but cigarette smoke induced a marked rise in lung in both TP and PN periods. The composite ratio of 3.2 for lung is thus driven by the cigarette smoke data. As indicated above, the potency difference in lung may be overstated by the tumor incidence data and in fact, there was greater malignancy from PN protocol (Balansky et al. 2012).

Discussion

In utero development is likely to be susceptible to genotoxics, and potentially other types of carcinogens, due to rapid cell division and long latency for tumor formation, similar to factors that make the early postnatal period also vulnerable (Barton et al. 2005; Ginsberg 2003). However, risk assessments do not typically take into account the risk associated with TP exposure to carcinogens. The 2009 National Academy of Sciences report titled *Science and Decisions* identified transplacental carcinogenesis as a key research area that needs incorporation into human health risk assessment (NRC (National Research Council) 2009). The simplest approach to accomplish this may be to extend the ADAFs developed for PN periods, or some alternative system of ADAFs, to the prenatal period. Whatever the selected approach, the existing body of evidence on TP carcinogenesis requires evaluation relative to other life stages. The current effort focused on the relationship between TP and PN vulnerability to genotoxic carcinogens tested under similar conditions.

The OEHHHA (California Office of Human Health Hazard Assessment) (2009a) database serves as an excellent starting point for the current analysis because it provides an extensive compilation of early life data and presents a statistical comparison of these results to adult-only exposures. The TP and PN relative potency estimates developed by OEHHHA are informative, but being a broad summary across numerous carcinogens, some of which were not tested in one or the other early life stage, it is not a direct comparison of potency for similarly tested compounds. The current analysis chose those carcinogens identified in the OEHHHA analysis that did have test data for both TP and PN life stages. The results for these nine agents were broken down by target organ and then reviewed with a 13-attribute tiering system to identify the most directly comparable TP and PN data for relative potency evaluation.

The tiering analysis found that most of the nine case-study carcinogens exhibit aspects that are suboptimal for side-by-side comparison of potency across life stages. Only one of the case studies was considered to be Tier 1 evidence, that for VC. Some of the reasons for lower weight of evidence (Tier 2) were changes in species, strain or dose route between life stages, or employing only a single dose level which was at maximum response such that there was limited indication of dose response. Most studies utilized a single dose level and thus may be difficult to decipher where one is on the dose-response curve if the response is

barely detectable on the one hand or maximal on the other. The main reason for a study to be placed into Tier 3 was the inability to estimate the dose level administered, a common problem in PN studies in which lactation is the source of exposure. In addition to the nine case-study carcinogens from the OEHHHA database, a case study involving cigarette smoke exposure was added due to its exemplary side-by-side testing of TP, PN vs adult-only exposure (Balansky et al. 2012). This study was given a Tier 1 rating.

The comparison of TP to PN potency for these 10 genotoxicant case investigations demonstrated that when weight-averaging across studies according to tier assignment, TP and PN periods appear to display similar vulnerability to early life carcinogenesis (Table 3, range of TP/PN ratios 0.69 to 5.9 with 5 of 6 comparisons within threefold of unity). The only exception in terms of target organs was the kidney which, based upon limited data, appears to exhibit greater susceptibility from TP than PN exposure. Exceptions at the individual study level are the markedly greater vulnerability of male liver to VC and 3-MC from PN as opposed to TP exposure. This may result from immaturity of activating enzymes in fetal liver, or a more efficient detoxification in the maternal system. The VC example is further informed by the enhanced response of kidney (Figure 4) and zymal gland (data not shown) to TP exposure, in spite of the lower response of liver. Data suggest that the immaturity of the fetal liver enables higher amounts of VC to reach other fetal tissues where activation and carcinogenesis occur. While VC and 3-MC represent cases of indirect acting carcinogens displaying low TP potency in liver, benzidine, safrole, and urethane are examples of moderate to high TP potency in liver for indirect acting carcinogens. Vulnerability to indirect acting carcinogens in early life likely depends upon the metabolic pathways involved, although urethane and VC both are activated by cytochrome *P*-450 (CYP)2E1 (Ghanayem 2006; Ghissassi et al. 1998) and yet displayed differing results from early life exposure. The other key difference may be the timing of exposure relative to maturity of metabolizing systems across the protocols and test animals used in the various assays.

There is a consistent pattern of heightened early life responsiveness for tissues that are slowly dividing in adults (liver, kidney, brain) with similar sensitivity to adults for rapidly dividing tissues (e.g., lung, bone marrow). This reinforces the concept that rapid rate of cell division is a major vulnerability factor for both TP and PN carcinogenesis and that carcinogens which are active at high dose on slowly dividing tissues in adults may be active at lower concentration in early life (Barton et al. 2005; Ginsberg 2003). Rapidly dividing tissues may still be subject to greater vulnerability from TP and PN exposure due to the greater latency period for the manifestation of tumors when exposure is in early life. The sex difference in liver response to early life carcinogen exposure (males having greater sensitivity than females, Figures 1 and 2) parallels the pattern seen in adult rodents as well as human liver (El-Serag 2012). This sex difference is believed to be related to the susceptibility to fatty liver disease (hepatic steatosis) and related metabolic disorders and inflammatory processes, factors which to some extent are under hormonal control (Cheung and Chen 2016; Naugler et al. 2007). Data suggest that promotional influences are important in manifesting liver cancer risk stemming from early life genotoxic exposure. The fact that the sex difference was observed in these early life studies suggests that even though the

animal early life database is limited it contains relevant information for understanding human cancer risk.

The clinical experience with DES, prescribed to pregnant women to prevent miscarriage and premature deliveries in the 1940s and 1950s, demonstrated that there is greater susceptibility to DES-mediated carcinogenesis from TP than adult exposure. It was reported that the relative risk for reproductive tract tumors in daughters of DES-exposed mothers was approximately 40-fold elevated (Troisi et al. 2007) with less elevated cancer risk in mothers themselves (Titus-Ernstoff et al. 2001; Dai et al. 2018a; 2018b). Although study comparability is an issue, DES animal experiments also provide evidence of greater susceptibility after developmental exposure than adult-only exposure for some cancer outcomes (Marselos and Tomatis 1992; Dai et al. 2018a; 2018b). Additional analyses are underway to assess the relative susceptibility to environmental and pharmaceutical estrogens, including DES, from developmental as compared to adult-only exposures in both animal and human investigations (Dai et al. 2018a; 2018b; Dai, personal communication). This effort may inform whether there is a need to extend the ADAF approach to estrogenic carcinogens.

Uncertainties

This analysis was constrained to the nine case-study carcinogens which were identified from the California OEHHA database that have TP, PN and adult-only data plus a Tier 1 dataset available from the more recent literature involving cigarette smoke. OEHHA (2009a) identified five additional carcinogens which have sufficient TP data to compare against adult-only exposure. However, those cases do not possess PN data and so one cannot perform the TP/PN comparison that was the primary goal of this assessment. In each of those five cases, the TP potency was less than from adult-only exposure and thus unlikely that those chemical examples will display greater potency from TP than from PN exposure. Our supplemental literature review (Supplement Table 10) found two additional genotoxicant case examples where data were available for TP, PN and adult animals thus enabling TP to PN potency comparison: the cigarette smoke study described above and 3-azido-3-deoxythymidine (AZT, Diwan et al. 1999; NTP 2006). The AZT studies included both TP and PN exposure groups but by different dose routes and without an adult-only comparison. A study on AZT by the National Toxicology Program (1999) focused on adult-only exposure but USEPA chose not to compile the Diwan/NTP data in its early life analysis due to protocol differences (U.S. Environmental Protection Agency (U.S. EPA) 2005; Barton et al. 2005). The AZT results are similar for TP and PN exposures showing liver and lung increases over control for doses ranging from 100 to 500 mg/kg/day over early life periods (Diwan et al. 1999; Toxicology 2006). Numerical increases in mammary tumors also occurred after both early life protocols. Thus, the AZT studies (Diwan et al. 1999; Toxicology 2006) may be considered to add a Tier 2 level of evidence supporting the concept that vulnerability of the TP and PN periods are similar for genotoxic carcinogens. This is the same overall conclusion drawn by the OEHHA (2009a) analysis which was somewhat greater but less specific in terms of a side-by-side comparison for specific carcinogens and target organs. Additional studies with genotoxicants and evaluation of TP carcinogenesis publications involving non-genotoxic mechanisms such as endocrine

modulation as exemplified by DES (Titus-Ernstoff et al. 2001), or other mechanisms as exemplified by inorganic arsenic (Waalkes et al. 2003) would help to bolster and extend the current results.

Some of the underlying studies had incomplete histopathology and did not report a combined tumor incidence across multiple sites. Thus, there are uncertainties in assessing the overall tumor rate following early life in relation to adult exposure. The OEHHHA (2009a) approach provided an overall “multisite potency distribution” stemming from each study which was an additive approach involving those tumors identified as statistically elevated. The early life to adult potency ratios reported by OEHHHA (2009a) using this multisite approach are similar to those noted presently for the main cancer target in the investigation suggesting that focusing on tumor response in the main target tissue did not markedly affect life stage comparison to a substantial degree.

Another uncertainty is whether cancer is observed in unique target organs after TP-only exposure. The best evidence for this in the current dataset is the kidney, an example which involves two genotoxicants, one at a Tier 1 level of evidence (Figure 4). However, as previously described this is still limited evidence and the kidney tumor response from TP exposure was 10% or less. The early life origins of kidney cancer in animals or humans has not been a research topic although evidence of a genetic component involving inheritance of a deficient Von Hippel-Lindau (VHL tumor suppressor) gene is consistent with an early life susceptibility (Ashouri et al. 2015). Prenatal genotoxicant exposure that produces a deficiency in the renal VHL gene may be a key predisposing factor for later emergence of renal cancer, with this genetic damage being more likely during a period of rapid growth and development than in the stable adult kidney. The lack of a PN kidney response in these two cases may be due to the rapid development of liver metabolizing enzymes which may prevent substantial amounts of chemical from reaching the kidney in the PN period.

There are design features in early life studies that vary considerably from study to study and which make comparing and combining across investigations uncertain. For example, in some TP studies, the dam was dosed throughout gestation while in others the prenatal exposure was on a single or several days. This is also the case for the PN period where dosing may have occurred throughout the juvenile period or on only for a portion thereof. The observation period between the start of chemical administration and sacrifice might also vary widely across investigations as well as numerous other features including number of animals per group, number of tissues assessed, dose route and magnitude of each dose. While the tiering approach was intended to weigh the analysis towards those case examples in which the TP and PN potencies are least uncertain and most comparable, most of the comparisons are of Tier 2 or Tier 3 caliber and therefore contain significant uncertainty. Thus, only general conclusions are possible at this time due to limited data. The fact that the patterns of response were similar between TP and PN exposure, such as male liver being more responsive than female liver or brain being highly responsive from both early life windows while blood-borne cancers were not, builds confidence in the conclusion from the quantitative analysis in Table 3 that differences in sensitivity between TP and PN are not substantial. However, there are notable exceptions as described above, which makes chemical-specific data of high value where it can be obtained.

Risk assessment implications

Evidence indicates that TP and PN windows of exposure are similarly vulnerable to genotoxic carcinogens (Table 3) which leads to the possibility of applying the 10-fold ADAF that is normally also reserved for the first 2 years of life to exposures during pregnancy. OEHHHA (2009b) similarly made this determination and applied a 10-fold ADAF to the last trimester of pregnancy, on the basis that the early PN period in rodents is akin to the last trimester of pregnancy. Further, later in pregnancy metabolic activation is more likely as fetal enzymes become more active. The available rodent TP investigations do not provide an empirical basis for a specific vulnerability window within pregnancy for carcinogenic effect, but the overall conclusion that susceptibility is similar during the TP and PN periods leaves open the question of whether the window of concern may be longer than only the third trimester of pregnancy. This window is difficult to further clarify due to the limited range of prenatal study designs and variability across chemicals and studies, as well as the inexact matching of early life windows of vulnerability across species. The focus on the last trimester of pregnancy is in step with the science-policy assumption made for risk calculations by OEHHHA (2009b), to explore the implications of this vulnerable life stage.

The OEHHHA approach of applying the 10-fold ADAF to the last trimester of pregnancy incrementally adds (approximately 2.5%) to the lifetime risk when combined with childhood and adult exposures. Thus, it may exert a greater impact on shorter term exposure scenarios such as when calculating risks involved in remediation projects that might take months to a few years. Another type of calculation is to estimate the dose during pregnancy that may result in an increased cancer risk of 1 in a million in the offspring. Such a target dose might form the basis for further risk evaluation and potentially lead to measures to reduce cancer risk during the sensitive prenatal life stage. The value of calculating the dose associated with a one in a million risk during this period is to determine whether this sensitive life stage may merit specific precautions. The following example uses a one in a million risk target and assumes the last trimester of pregnancy is the susceptible period for TP carcinogenesis although the length of this vulnerable period is highly uncertain and may differ with different carcinogens and target sites. Although a one in a million risk is often referred to as 'de minimis', when this level of risk results from a three-month exposure, it needs to be acknowledged as substantial.

The flame retardant tris-(1,3-dichloro-2-propyl phosphate) (TDCPP) serves as an illustration of the less than lifetime approach to evaluate TP risk (Figure 8). TDCPP is a mutagen and clastogen which has liver, kidney, and testes as cancer target sites (Gold, Blum, and Ames 1978; Freudenthal and Henrich 2000). At least two of these sites (liver and kidney) show heightened vulnerability from TP exposure. Thus, TDCPP is a useful case study to exemplify risk during pregnancy. OEHHHA derived an adult-based cancer slope factor of 0.13/mg-kg-d for TDCPP. The current analysis suggests that with regards to TP cancer, a 10-fold ADAF may be applied to the last trimester of pregnancy, increasing the slope factor during this vulnerable period to 1.3/mg-kg-d. However, the TDCPP slope was based upon lifetime exposure and thus to calculate the daily increment of risk during pregnancy this pregnancy risk is pro-rated over the entire lifespan: $1.3/\text{mg-kg-d} \times 3 \text{ months}/(12 \times 70) = 4.6\text{E-}03/\text{mg-kg-d}$ for the third trimester of pregnancy. This prorated slope factor may then be

employed to calculate the daily dose during the third trimester that would be associated with a 1 per million cancer risk: $2.2\text{E-}04$ mg/kg/d. This can be a guide to TDCPP exposures during pregnancy, a target dose that might be considered best not to exceed to prevent an elevated TP cancer risk.

Exposure to TDCPP is common as it is in numerous consumer items. The National Academy of Sciences calculated the daily dose from adult contact with furniture containing TDCPP to be $2.6\text{E-}03$ mg/kg/d (NRC 2000). More recent urinary biomonitoring data from pregnant women in Rhode Island suggest a median daily exposure of $5\text{E-}05$ mg/kg/d and a maximum of $1.4\text{E-}03$ mg/kg/d (Romano et al. 2017). The latter estimate is based upon conversion of urinary TDCPP metabolite levels to intake dose from % dose excreted in urine as the biomonitored metabolite and standard assumptions regarding urinary volume and body weight during pregnancy (Health Canada 2016). These various exposure estimates suggest that $1.4\text{E-}03$ mg/kg/d is a plausible upper bound exposure estimate. This is sixfold greater than the third trimester daily dose associated with a 1 per million cancer risk, suggesting that general population exposures of TDCPP may be associated with a TP cancer risk that merits further evaluation. A longer term scenario involving pregnancy and postnatal exposures together, which is likely for exposure to home furnishings and house dust, would involve a greater pro-rated slope factor and thus a lower daily dose associated with a 1 per million risk from these exposures.

Summary

An in-depth evaluation of the nine carcinogens identified by OEHHHA (2009a) as having both TP and PN exposure suggests similarities in vulnerability between these two periods of early life exposure and that it is reasonable to apply a 10-fold ADAF to exposures during some portion or all of pregnancy. Given that TP vulnerability to genotoxic carcinogens might be observed in the limited dataset available, this emphasizes the need to apply ADAFs to adjust for potential increased potency when chemical-specific data are not available. The tumor response in terms of sex, target organ and chemical is similar across these two lifestages with the degree of susceptibility relative to adults also similar in most cases. This agrees with the OEHHHA assessment of the overall dataset. The strongest support for this approach is for genotoxic carcinogens capable of inducing cancer in liver, brain, kidney and perhaps other organs that are slowly dividing in adults but rapidly dividing *in utero*. However, additional research into non-genotoxic TP carcinogens such as estrogen agonists and arsenic may reveal the need to broaden this approach to additional cancer mechanisms of action. This vulnerability window might have the greatest impact on short-term risk assessments, with the adjusted “third trimester” potency useful also to calculate a third trimester dose resulting in a 1 per million cancer risk. This approach is exemplified with a mutagenic carcinogen flame retardant to which there is common human exposure. The current analysis provides a means to analyze such short-term cancer risks from prenatal exposures which might complement the addition to longer term and lifetime cancer risk assessment.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

- Ashouri K, Mohseni S, Tourtelot J, Sharma P, and Spiess PE. 2015 Implications of Von Hippel-Lindau syndrome and renal cell carcinoma. *J Kidney Cancer* 2:163–73. doi:10.15586/jkcvhl.2015.41.
- Autrup H 1993 Transplacental transfer of genotoxins and transplacental carcinogenesis. *Environ Health Perspect* 101 (Suppl 2):33–38. doi:10.1289/ehp.93101s233.
- Balansky R, Ganchev G, Ilcheva M, Nikolov M, Steele VE, and De Flora S. 2012 Differential carcinogenicity of cigarette smoke in mice exposed either trans-placentally, early in life or in adulthood. *Int J Cancer* 130:1001–10. doi:10.1002/ijc.26103. [PubMed: 21484788]
- Barton HA, Coglian VJ, Flowers L, Valcovic L, Setzer RW, and Woodruff TJ. 2005 Assessing susceptibility from early-life exposure to carcinogens. *Environ Health Perspect* 113:1125–33. doi:10.1289/ehp.7667. [PubMed: 16140616]
- Botsivali M, and Kyrtopoulos SA. 2019 Tranplacental exposure to carcinogens and risk to children: Evidence from biomarker studies and the utility of omic profiling. *Arch Toxicol* 93:833–57. doi:10.1007/s00204-019-02428-3. [PubMed: 30859261]
- Brent RL 2014 Carcinogenic risks of prenatal ionizing radiation. *Semin Fetal Neonatal Med* 19:203–13. doi:10.1016/j.siny.2013.11.009. [PubMed: 24378676]
- Bunin GR, Kuijten RR, Boesel CP, Buckley JD, and Meadows AT. 1994 Maternal diet and risk of astrocytic glioma in children: A report from the Children's Cancer Group (United States and Canada). *Cancer Causes Control* 5:177–87. [PubMed: 8167265]
- Cheung OKW, and Cheng ASL. 2016 Gender differences in adipocyte metabolism and liver cancer progression. *Front Genet* 20. doi:10.3389/fgene.2016.00168.
- Cohn BA, Wolff MS, Cirillo PM, and Sholtz RI. 2007 DDT and breast cancer in young women: New data on the significance of age at exposure. *Environ Health Perspect* 115:1406–14. doi:10.1289/ehp.10260. [PubMed: 17938728]
- Cohn BA, Wolff MS, Cirillo PM, Sholtz RI, Cohn BA, La Merrill M, Krigbaum NY, Yeh G, Park JS, Zimmermann L, et al. 2015 DDT exposure in utero and breast cancer. *J Clin Endocrinol Metab* 100:2865–72. doi:10.1210/jc.2015-1841. [PubMed: 26079774]
- Dai M, Euling SY, and Foos B. 2018a A comparative approach to assessing lifestage-dependent susceptibility to estrogenic agent exposures and cancer development. International Conference on Prenatal Programming and Toxicity (PPTOX VI) Tórshavn, Faroe Islands Poster # P030.
- Dai M, Euling SY, and Foos B. 2018b Does Early-Life Exposure to Estrogenic Chemicals Warrant an Age Dependent Adjustment Factor in Carcinogen Risk Assessments? Teratology Society 58th Annual Meeting, Clearwater, FL Poster Abstract P60, published in *Birth Defects Research Journal* doi:10.1002/bdr2.1355.
- Diwan BA, Riggs CW, Logsdon D, Haines DC, Olivero OA, Rice JM, Yuspa SH, Poirier MC, and Anderson LM. 1999 Multiorgan transplacental and neonatal carcinogenicity of 3-azido-3'-deoxythymidine in mice. *Toxicol Appl Pharmacol* 16:82–99. doi:10.1006/taap.1999.8782.

- Druckrey H, and Landschutz C. 1971 Transplacental and neonatal carcinogenesis by ethylnitrosobiuret (enbu) in bd ix-rats. *Z Krebsforsch Klin Onkol Cancer Res Clin Oncol* 76 (1):45–58. [PubMed: 4329184]
- El-Serag HB 2012 Epidemiology of viral hepatitis and hepatocellular carcinoma. *Gastroenterology* 142:1264–73. doi:10.1053/j.gastro.2011. [PubMed: 22537432]
- Freudenthal RI, and Henrich RT. 2000 Chronic toxicity and carcinogenic potential of tris-(1,3-dichloro-2-propyl) phosphate in Sprague-Dawley rats. *Int J Toxicol* 19:119–25. doi:10.1080/109158100224926.
- Ghanayem BI 2006 Inhibition of urethane-induced carcinogenicity in Cyp2E1^{-/-} in comparison to Cyp2E1^{+/+} mice. *Toxicol Sci* 95:331–39. doi:10.1093/toxsci/kfl158. [PubMed: 17093202]
- Ghissaassi FE, Bartsch H, and Barbin A. 1998 Metabolic activation of vinyl chloride by rat liver microsomes: Low dose kinetics and involvement of cytochrome P450 2E1. *Biochem Pharmacol* 55:1445–52. doi:10.1016/s0006-2952(97)00645-x. [PubMed: 10076537]
- Ginsberg GL 2003 Assessing cancer risks from short-term exposures in children. *Risk Anal* 23:19–34. [PubMed: 12635720]
- Gold MD, Blum A, and Ames BN. 1978 Another flame retardant, tris-(1,3-dichloro-2-propyl)-phosphate, and its expected metabolites are mutagens. *Science* 200:785–87. doi:10.1126/science.347576. [PubMed: 347576]
- Health Canada. 2016 Draft screening assessment, certain organic flame retardants substance grouping: TCPP 13674–84-5 and TDCPP 13674-87-8. Appendix F: TDCPP intake estimate from urinary BDCPP biomonitoring reverse dosimetry. Accessed. <http://www.ec.gc.ca/ese-ees/default.asp?lang=En&n=B4374491-1>
- International Agency for Research on Cancer (IARC) IARC Monographs on the Evaluation of Carcinogenic Risks to Humans. 2010 Volume 94: Ingested nitrates and nitrites, and cyanobacterial peptide toxins. Lyon, France.
- Klein M 1959 Influence of low dose of 2-acetylaminofluorene on liver tumorigenesis in mice. *Proc Soc Exp Biol Med* 101:637–8. [PubMed: 14409691]
- Kommineni VR, Greenblatt M, Mihailovich N, and Vesselinovitch SD. 1970 The significance of perinatal age periods and the dose of urethan on the tumor profile in the mrc rat. *Cancer Res* 30 (10):2552–5. [PubMed: 4319761]
- Maltoni C, Lefemine G, Ciliberti A, Cotti G, and Carretti D. 1981 Carcinogenicity bioassays of vinyl chloride monomer: a model of risk assessment on an experimental basis. *Environ Health Perspect* 41:3–29. [PubMed: 6800782]
- Marselos M, and Tomatis L. 1992 Diethylstilboestrol: II, pharmacology, toxicology and carcinogenicity in experimental animals. *Eur J Cancer* 29A:149–55. doi:10.1016/0959-8049(93)90597-9. [PubMed: 1445734]
- McKean-Cowdin R, Pogoda JM, Lijinsky W, Holly EA, Mueller BA, and Preston-Martin S. 2003 Maternal prenatal exposure to nitrosatable drugs and childhood brain tumours. *Int J Epidemiol* 32:211–17. doi:10.1093/ije/dyg050. [PubMed: 12714539]
- Miller MS 2004 Transplacental lung carcinogenesis: Molecular mechanisms and pathogenesis. *Toxicol Appl Pharmacol* 198:95–110. doi:10.1016/j.taap.2003.07.018. [PubMed: 15236948]
- Mohr U, Emura M, Kamino K, Steinmann J, Kohler M, and Morawietz G, et al. 1995 Increased risk of cancer in the descendants of syrian hamsters exposed prenatally to diethylnitrosamine (den). *Int J Cancer* 63 (1):86–91. [PubMed: 7558458]
- Naito M, Naito Y, and Ito A. 1981 Effect of age at treatment on the incidence and location of neurogenic tumors induced in Wistar rats by a single dose of N-ethyl-N-nitrosourea. *Gann* 72:569–77. [PubMed: 7308669]
- Naugler WE, Sakurai T, Kim S, Maeda S, Kim K, Elsharkawy AM, and Karin M. 2007 Gender disparity in liver cancer due to sex differences in MyD88-dependent IL-6 production. *Science* 317:121–24. doi:10.1126/science.1140485. [PubMed: 17615358]
- Nielsen S, Mueller BA, Preston-Martin S, Farin FM, and Holly EA, McKean-Cowdin R. *Cancer Epidemiol Biomarkers Prev*. 2011 11;20(11):2413–9. doi:10.1158/1055-9965.EPI-11-0196. Epub 2011 Sep [PubMed: 21914837]

- Nomura T 1976 Comparison of tumour susceptibility among various organs of foetal. Young and Adult Icr/jcl Mice. *Br J Cancer* 33 (5):521–34. [PubMed: 179560]
- NRC (National Research Council). 2000 Toxicological risks of selected flame retardant chemicals. Washington DC: National Academy Press Accessed. <https://www.nap.edu/catalog/9841/toxicological-risks-of-selected-flameretardant-chemicals>
- NRC (National Research Council). 2009 Science and decisions: Advancing risk assessment. Washington DC: National Academy Press.
- NTP toxicology and carcinogenesis studies of AZT (CAS No. 30516–87-1) and AZT/alpha-Interferon A/D B6C3F1 Mice (Gavage Studies). 1999 Natl Toxicol Program Tech Rep Ser.
- OEHHA (California Office of Environmental Health Hazard Assessment) 2009a In utero and early life susceptibility to carcinogens. Accessed. <https://oehha.ca.gov/media/downloads/crn/appendixyearly.pdf>
- OEHHA (California Office of Environmental Health Hazard Assessment) 2009b Technical support document for cancer potency factors, 2009. Accessed: <https://oehha.ca.gov/air/crn/technical-support-document-cancer-potencyfactors-2009>
- Preston-Martin S 1989 Epidemiological studies of perinatal carcinogenesis IARC Sci Publ 96:289–314. International Agency for Research on Cancer, Lyon France.
- Rice JM 1981 Prenatal susceptibility to carcinogenesis by xenobiotic substances including vinyl chloride. *Environ Health Perspect* 41:179–88. doi:10.1289/ehp.8141179. [PubMed: 7333236]
- Romano ME, Hawley NL, Eliot M, Calafat AM, Jayatilaka NK, Kelsey K, McGarvey S, Phipps MG, Savitz DA, Werner EF, et al. 2017 Variability and predictors of urinary concentrations of organophosphate flame retardant metabolites among pregnant women in Rhode Island. *Environ Health* 16:40. doi:10.1186/s12940017-0247-z. [PubMed: 28399857]
- Searles-Nielsen S, Mueller BA, Preston-Martin S, Farin FM, Holly EA, and McKean-Cowdin R. 2011 Childhood brain tumors and maternal cured meat consumption in pregnancy: Differential effect by glutathione S-transferases. *Cancer Epidemiol Biomarkers Prev* 20:2413–19. doi:10.1158/1055-9965.EPI-11-0196. [PubMed: 21914837]
- Titus-Ernstoff L, Hatch EE, Hoover RN, Palmer J, Greenberg ER, Ricker W, Kaufman R, Noller K, Herbst AL, Colton T, et al. 2001 Long-term cancer risk in women given diethylstilbestrol (DES) during pregnancy. *Br J Cancer* 84:126–33. doi:10.1054/bjoc.2000.1521. [PubMed: 11139327]
- Tomatis L, Turusov V, Guibbert D, Duperray B, Malaveille C, and Pacheco H. 1971 Transplacental carcinogenic effect of 3-methylcholanthrene in mice and its quantitation in fetal tissues. *J Natl Cancer Inst* 47(3):645–51. [PubMed: 5157583]
- Toxicology NTP 2006 Carcinogenesis Studies of AZT (in utero studies) in Swiss Mice. *Ntp Tr* 522.
- Troisi R, Hatch EE, Titus-Ernstoff L, Hyer M, Palmer JR, Robboy SJ, Strohsnitter WC, Kaufman R, Herbst AL, and Hoover RN. 2007 Cancer risk in women prenatally exposed to diethylstilbestrol. *Int J Cancer* 121:356–60. doi:10.1002/ijc.22631. [PubMed: 17390375]
- Turusov V, Tomatis L, Guibberti D, Duperray B, and Pacheco H. (1973). The effect of prenatal exposure of mice to methyl cholanthrene combined with the neonatal administration of diethylnitrosamine. In: Tomatis L And Mohr U (ED.). International Agency for Research on Cancer Scientific Publication, No 4. Transplacental Carcinogenesis. Proceeding of a Meeting, Hannover, West Germany, Oct 6-7, 1971 XII pp. 84–91. International Agency for Research on Cancer: Lyon, France.
- U.S. Environmental Protection Agency (U.S. EPA). 2005 Supplemental guidance for assessing cancer susceptibility from early-life exposure to carcinogens. EPA/630/R-03/003F. 3.
- Vesselinovitch SD, Rao KV, and Mihailovich N. 1975 Factors modulating benzidine carcinogenicity bioassay. *Cancer Res* 35(10):2814–9. [PubMed: 1157051]
- Vesselinovitch SD, Rao KV, and Mihailovich N. 1979 Transplacental and lactational carcinogenesis by safrole. *Cancer Res* 39 (11):4378–80. [PubMed: 498070]
- Vesselinovitch SD, Koka M, Mihailovich N, and Rao KV. 1984 Carcinogenicity of diethylnitrosamine in new-born. Infant, and Adult Mice. *J Cancer Res Clin Oncol* 108 (1):60–5. [PubMed: 6746718]
- Waalkes MP, Ward JM, Liu J, and Diwan BA. 2003 Transplacental carcinogenicity of inorganic arsenic in the drinking water: Induction of hepatic, ovarian, pulmonary, and adrenal tumors in mice. *Toxicol Appl Pharmacol* 186:7–17. doi:10.1016/S0041-008X(02)00022-4. [PubMed: 12583988]

Zeller WJ, Ivankovic S, and Zeller J. 1978 Induction of malignant tumors in Wistar and Sprague-Dawley rats by single doses of n-butyl-nitrosourea in perinatal and juvenile phases of development. Arch Geschwulstforsch 48:9–16. [PubMed: 566089]

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Figure 1.

TP/PN Comparisons for Liver (Male). Cancer potency estimates for early life stages are compared to adult-only exposure or compared between early life stages as indicated in the figure. Abbreviations: TP – transplacental; PN – postnatal. Each study and database were categorized into Tier 1, 2 or 3 levels of evidence as described in text. Supplement tables contain chemical-specific data.

Figure 2.
TP/PN Comparisons for Liver (Female). See Figure Legend 1 for description and abbreviations.

Figure 3.
TP/PN Comparisons for Brain (sex combined or not specified in study). See Figure Legend 1 for description and abbreviations.

Figure 4.
TP/PN Comparisons for Kidney (sex combined or not specified in study). See Figure Legend 1 for description and abbreviations.

Transplacental:Postnatal Comparisons: Lung

Figure 5.
TP/PN Comparisons for Lung. See Figure Legend 1 for description and abbreviations.

Transplacental:Postnatal Comparisons: Leukemia / Lymphoma

Figure 6.

TP/PN Comparisons for Leukemia/Lymphoma (sex combined or not specified in study). See Figure Legend 1 for description and abbreviations. TP to PN ratio not shown because neither early life period exhibited a significant response.

Figure 7.
Cigarette smoke-induced lung tumors in mice exposed early in life vs adult only. See Figure Legend 1 for description and abbreviations.

Estimation of Cancer Risk from Prenatal Exposure: Case Example with TDCPP

Figure 8.

Estimation of cancer risk from prenatal exposure: case example with TDCPP.

Table 1.

Attributes for determining evidence tiers for life stage carcinogenicity studies.

Attribute	Description	Importance
I	At least one study in a chemical's database contains a start/stop exposure pattern focusing on an early life period	Core
II	Database also contains older juvenile (at least one month of age) or adult only dosing	Core
III	Appropriate controls in each age group or at least a well conducted common control group that matches the full age range of dosing and observation	Core
IV	Test chemical was a single agent or well defined mixture that is same across age groups	Core
V	Reporting of age dosed, age at sacrifice and tumor incidence	Core
VI	Dose administered on each day of dosing is known or can be calculated	Key
VII	Comparable dose routes and dose levels across life stages	Key
VIII	Same species and strain across age groups	Key
IX	Dose level set so no overt toxicity or not yielding a maximal tumor response	Key
X	At least one year latency from start of dosing	Key
XI	Sufficient numbers of animals in each group (e.g., at least 10) (KEY if negative results, DESIREABLE if positive results)	Key/Desireable
XII	More than one dose level in each age group	Desireable
XIII	Full histopathology analysis of tissues	Desireable

Table 2.

Study design and tiering for lifestage studies^a.

Chemical	Transplacental (TP)	Postnatal (PN)	Comments
Benzidine	Daily via diet – mouse	Daily until weaning via lactation- mouse	Tier III – Dissimilar route and PN dose uncertain due to lactation
BNU	Single injection; Wistar rat but “adult” is SD rat	Two days of injection; Wistar but adult is SD	Tier II – strain switch in “adult” and this is actually Day 30 juvenile, not true adult
Cigarette smoke	Daily inhalation – mice	Daily inhalation post-natal days 1–120	Tier I – same study design and laboratory across TP, PN and adult only exposures
DEN	Single day gavage – hamster	Multiple gavage days – mice	Tier II – Species and frequency of dosing differs between TP and PN
ENB	Single day gavage – rats	Single day sc injection – rats	Tier II – dose route different between TP and PN
ENU	Single day injection – rats	Single day inj – rats	Tier II – no adult data but adult rate of key target (brain tumors) estimated from Rice 1981
3-MC	Single day gavage – CFI mice	Repeat gavage – albino mice	Tier II – TP and PN run in different labs, different mouse strains, different dosing frequency
Safrole	Gavage every other day of gestation – mouse	Dose to dam, exposure via breast milk – mouse	Tier III – PN dose unknown due to lactational exposure
Urethane – mice	Daily injection	Daily until weaning via lactation	Tier III – PN dose unknown due to lactational exposure
Urethane – rats	Single day ip	Repeat dose ip	Tier II – TP and PN protocols similar but tumor response marginal at main site (liver, 2/44) making significance uncertain
VC	Daily inhalation – rats	Repeat dose inhalation – rats	Tier I – multiple dose levels, similar protocol across TP, PN and adult

^aFor more bioassay details see Appendix tables. References and abbreviations are as follows; Benzidine – Vesselinovitch et al. 1975; BNU (butylnitrosourea) – Zeller, Ivankovic, and Zeller (1978); DEN (diethylnitrosamine) – Mohr et al. 1995 (TP); Vesselinovitch et al. 1984 (PN); ENB (ethylnitrosobutiret) – Druckery and Landschutz 1971; ENU (ethylnitrosourea) – Naito, Naito, and Ito (1981); 3-MC (3-methylcholanthrene) – Turusov et al. 1973; Klein et al. 1959; Tomatis et al. 1971; Safrole: Vesselinovitch et al. 1979; Urethane (mice) – Nomura et al. 1976; Urethane (rats) Komminen et al. 1970; VC (vinyl chloride) – Maltoni et al. 1981.

Table 3.

Tier-Weight Averaging of TP/PN Potency Ratio. Each row shows the ratio of TP/PN potency for a given target organ according to level of evidence (Tier 1, 2, or 3). The final column shows the tier-weight averaged ratio as described in Methods.

Target Organ	Tier 1 (100% weight)	Tier 2 (50% weight)	Tier 3 (25% weight)	Weighted TP/PN
Liver Male or M/F combined	Vinyl chloride: 0.03	Urethane rat: 2.3 3-MC: 0.12	Urethane mouse: 2.7 Saffrole: 0.49 Benzidine: 0.74	0.81
Liver Female	-	3-MC: 0.07	Saffrole: 1.2 Benzidine: 1.4	0.69
Brain	-	BNU: 0.6 ENB: 2.3	ENU: 1.75	1.5
Kidney	Vinyl chloride: 4.8	BNU: 8	-	5.9
Lung	Cigarette smoke: 5.4	Urethane: 0.96 DEN: 0.84	-	3.2