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Assessing Cancer Risks from Short-Term Exposures in Children

Gary L. Ginsberg*

For the vast majority of chemicals that have cancer potency estimates on IRIS, the underlying database is deficient with respect to early-life exposures. This data gap has prevented derivation of cancer potency factors that are relevant to this time period, and so assessments may not fully address children's risks. This article provides a review of juvenile animal bioassay data in comparison to adult animal data for a broad array of carcinogens. This comparison indicates that short-term exposures in early life are likely to yield a greater tumor response than short-term exposures in adults, but similar tumor response when compared to long-term exposures in adults. This evidence is brought into a risk assessment context by proposing an approach that: (1) does not prorate children's exposures over the entire life span or mix them with exposures that occur at other ages; (2) applies the cancer slope factor from adult animal or human epidemiology studies to the children's exposure dose to calculate the cancer risk associated with the early-life period; and (3) adds the cancer risk for young children to that for older children/adults to yield a total lifetime cancer risk. The proposed approach allows for the unique exposure and pharmacokinetic factors associated with young children to be fully weighted in the cancer risk assessment. It is very similar to the approach currently used by U.S. EPA for vinyl chloride. The current analysis finds that the database of early life and adult cancer bioassays supports extension of this approach from vinyl chloride to other carcinogens of diverse mode of action. This approach should be enhanced by early-life data specific to the particular carcinogen under analysis whenever possible.

KEY WORDS: Cancer; risk assessment; children

1. INTRODUCTION

The general lack of cancer dose-response data in animals exposed at early-life stages or in children has been an obstacle to developing guidelines that address cancer risk in children (U.S. EPA, 1999). The standard rodent cancer bioassay begins when animals are sexually mature young adults, typically at five to six weeks of age. Thus, for the vast majority of chemicals for which cancer slope and unit risk factors are available on IRIS, the underlying bioassay database is deficient with respect to early-life exposures. This data gap precludes the derivation of cancer potency factors that take into account early-life exposures and so may not fully address children's risks. However, it may be possible to derive a generic approach to assessing children's cancer risks based on what is known for the limited number of chemicals that have been tested in juvenile rodents.

The goal of this research is to summarize and analyze a variety of cancer bioassay data sets that include juvenile animal exposures to compare carcinogen sensitivity between neonatal and adult-only exposures. Also reviewed are mechanistic considerations that pertain to children's sensitivity. Based on this analysis, an approach for adjusting cancer risk assessment

* Address correspondence to Gary L. Ginsberg, Ph.D., Connecticut Department of Public Health, Division of Environmental Epidemiology & Occupational Health, PO Box 340308, MS 11CHA, Hartford, CT 06134-0308; gary.ginsberg@po.state.ct.us.

methodologies to take into account children's exposures is proposed.

A particular focus is on relatively short-term exposures in early life since current methodologies simply prorate the exposure over the entire life span without considering the possibility of differential sensitivity in early life. Since this tends to minimize the impact of unique exposure (U.S. EPA, 2000a) and pharmacokinetic (Ginsberg *et al.*, 2002; Renwick, Dorne, & Walton, 2000) factors that occur during childhood, this assumption needs close inspection. The one exception to this approach is the recent vinyl chloride cancer risk assessment in which U.S. EPA indicates that it is inappropriate to prorate childhood exposures and risks over the entire life span for this carcinogen (U.S. EPA, 2000b). The current analysis reviews this evidence for vinyl chloride as well as for a number of other carcinogens to see if U.S. EPA's recommended approach for vinyl chloride may be more broadly applicable. This article is an effort to move beyond previous reviews of juvenile animal carcinogenicity data (Toth, 1968; Vesselinovitch, Rao, & Mihailovich, 1979; McConnell, 1992; Anderson *et al.*, 2000) by placing these data into a risk assessment context that may be useful for evaluating cancer risk in children.

2. THEORETICAL BASIS: MODE OF ACTION CONSIDERATIONS IN EARLY LIFE

2.1. Genotoxic Carcinogens

The scientific rationale for a greater susceptibility in early life-stages comes from a consideration of the mode of action of genotoxic carcinogens. In this case, the key instigating event is reaction of carcinogen (whether parent compound or reactive metabolite) with DNA leading to modification of the template ahead of the next round of cell division. Examples of this modification are DNA adducts and DNA-protein cross-links (Garner, 1998). If the template is not repaired prior to the next round of replication, these modifications can be converted into point mutations or larger structural damage in DNA. These changes can in turn lead to tumorigenesis due to inappropriate DNA expression, for example, activation of oncogenes or inactivation of tumor suppressor genes.

The aspect of tumorigenesis that is essential to early life stages is the temporal relationship between DNA repair and cell division. If DNA repair is efficient and occurs first, cell division can occur in the absence of adducted or cross-linked DNA. However, if cell division occurs before DNA repair is complete, then the consequences outlined above can occur. Thus, genotoxic carcinogens are generally more effective in rapidly dividing tissues because the higher rate of cell division leaves less time for DNA repair prior to fixation of the damage as a mutation (Swenberg, Fedtke, & Fishbein, 1992; Anderson *et al.*, 2000). This appears to be one reason that tissues such as stomach, lung, skin, and bladder are common carcinogen targets, while more quiescent organs, such as the liver and brain, are less common targets.

Another manner rapid cell division can increase cancer risk is due to increased opportunities for carcinogen/DNA interaction. DNA adduct studies in rodent liver show that juvenile animals form more adducts than mature animals, with the amount of adduction greatest during the period of greatest hepatocyte proliferation (Laib *et al.*, 1989; Coccia *et al.*, 1988). This is consistent with the concept that cell division is a vulnerable time for DNA adduct formation, and that those regions that are being actively transcribed are more likely to be modified (Xu, Manning, & Patierno, 1994; Irwin & Wogan, 1984; Tomale *et al.*, 1994). The corollary to this is that in tissues undergoing extensive cell division, genes that promote cell growth are more actively transcribed and thus are more likely to be damaged by carcinogens. This concept is supported by data showing increased expression of a variety of oncogenes under conditions that stimulate cell proliferation (Goldsworthy *et al.*, 1994; Rosenwald *et al.*, 1995). The fact that DNA repair also occurs more rapidly in actively transcribed DNA can be a mitigating factor for some types of adducts that are readily repaired (Engelbergs *et al.*, 1998).

The rapid rate of growth in utero and during infancy leads to greater cell division rates even in normally quiescent tissues. This factor can be seen as predisposing to carcinogenesis for the rationale described above, which is in addition to the way cell proliferation is thought to promote tumorigenesis in multistage models (Moolgavkar, 1995). In the latter case, high rates of cell proliferation coming after an initiating (e.g., mutational or DNA damaging) event increases the probability that the initiated clone will expand before cell death (e.g., apoptosis) removes the altered cell(s). This expansion increases the probability of a second mutational event, which would result in loss of growth control and the transformed phenotype. This promotional mechanism may also contribute to children's susceptibility to tumorigenesis,

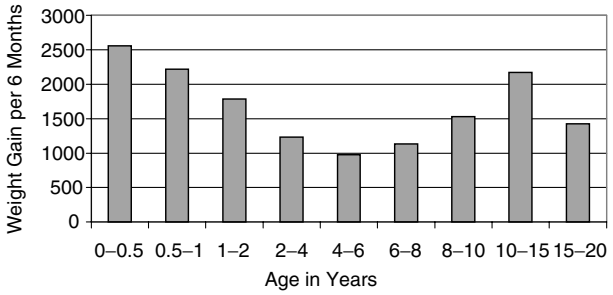

Fig. 1A. Change in body weight (g) with age (derived from equations in Haddad *et al*., 1999).

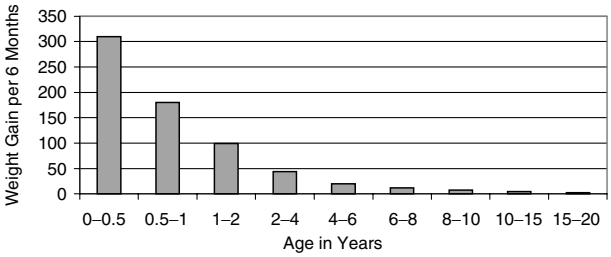

Fig. 1C. Change in brain weight (g) with age (derived from equations in Haddad *et al*., 1999).

leading to the possibility that both the initiating and promoting stages of tumorigenesis are enhanced in early life. An additional factor causing greater concern for early-life exposures is the greater latency period available for the conversion of the initiated state into actual tumors.

There are few measurements of the rate of cell division in rapidly growing tissues. However, this rate can be expected to be a function of organ growth. Fig. 1 shows human growth curves for body weight, liver weight, and brain weight as the rate of change per six-month time period. These figures are derived from the data published by Altmann and Ditmer (1962) and the growth curve equations derived from that data set by Haddad, Restieri, and Krishnam (1999). The figure shows rapid body weight gain in newborns, with decreasing weight gains over the following several years to a nadir at four to six years of age. This is followed by a period of increasing weight gain rates through adolescence that tail off in the late teens where body weight stabilizes as one approaches adulthood. The profile for liver weight gain is similar in that the highest rates are in early post-natal life, decrease thereafter, and then rebound in late childhood (Fig. 1B).

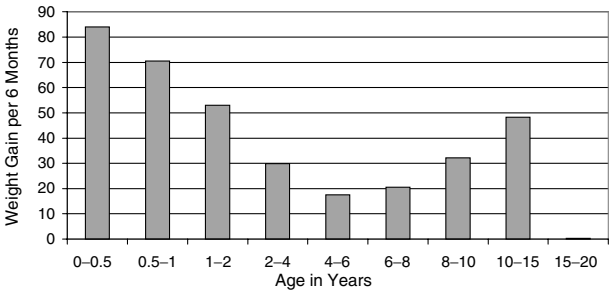

Fig. 1B. Change in liver weight (g) with age (derived from equations in Haddad *et al*., 1999).

However in this case, the difference between neonates and older groups is larger and growth is minimal beyond 15 years of age. The growth curve for kidney is similar to that for liver (data not shown). However, the profile for increasing brain weight (Fig. 1C) differs in that there is a continuous decline following the neonatal period, with the growth rate differential between neonates and older groups quite large. In this case, organ growth essentially ceases beyond age eight. Finally, as shown in curves presented in a National Academy of Science report, increases in testes and uterine weight are minor in early life with a dramatic rise occurring between 8 and 17 years of age (NAS, 1993).

These data suggest that the rate of cell division in brain, liver, and kidney is considerably greater during the first two years of life than in later childhood or in adults. This is supported by rodent studies that found high rates of DNA synthesis in rat liver during the first week of life in comparison to adult rat liver, in which DNA synthesis is extremely slow (Leakakos & Shank, 1994; Timchenko *et al.*, 1997; Craddock, 1976; Coccia *et al.*, 1988). This period of rapid proliferation is when the liver is most sensitive to DNA adduction and hepatocarcinogenesis caused by urethane (Nomura, 1976), vinyl chloride (Laib, Klein, & Bolt, 1985; Laib *et al.*, 1989), and dimethylnitrosamine (Coccia *et al.*, 1988). It is not clear whether the second peak in organ growth for human liver seen beginning at age six (Fig. 1) is due to a new wave of cell proliferation or due to the existing cells becoming larger. However, it appears that the period of highest susceptibility relative to adults for quiescent tissues such as liver, kidney, and brain can extend over several years of early childhood. In this regard, it is noteworthy that clinical and epidemiological evidence point to tumors of the CNS and kidney (Wilm's tumor) as particular concerns in childhood (Schmidt, 1998; Anderson *et al.*,

2000). The period of cell-division-related vulnerability for reproductive tissues may instead be just before and during the pubertal period.

2.2. Indirect-Acting and Nongenotoxic Carcinogens

The above description has been confined thus far to genotoxic carcinogens, those whose mode of action involves direct interaction of parent compound or metabolite with DNA. However, carcinogens may also be genotoxic via indirect means involving modification of DNA through oxidative damage or radical generation. These mechanism may also produce a greater risk during periods of rapid cell proliferation. As with direct-acting genotoxicants, the proliferative state may leave DNA more accessible to oxidative/radical attack and leave less opportunity for DNA repair prior to fixation of the damage as a mutation or chromosomal rearrangement. This may cause periods of rapid growth in early life to also be more susceptible to these types of carcinogens. Of course, a variety of other factors may differ between children and adults (balance between metabolic activation and detoxification; ability of cellular defenses to compensate for oxidative stress and repair damage) that could affect the outcome of exposure during childhood. The uncertainty caused by child/adult toxicokinetic differences is addressed together with other uncertainties in Section 4.3.

This raises the question of whether other carcinogenic mechanisms (nongenotoxic) might also be more active in the developing organism. An important group to consider are agents that alter hormonal status or in other ways modify DNA expression to affect the balance between cell division and cell differentiation (e.g., estrogens, TCDD, PCBs). Such promoters are generally thought to require long-term exposures to yield tumors. However, it is possible that heightened windows of vulnerability to these agents may exist during early life or puberty, thus allowing short-term exposures to confer a significant cancer risk. The evidence that estradiol was positive in the newborn mouse model while phenobarbital was negative indicates that the specific promotional mechanism may govern whether developing organisms are sensitive (Flammang *et al.*, 1997; Fujii, 1991). This article will summarize data for a variety of other promoters (DES, tamoxifen, DDT, dieldrin) that indicate early-life sensitivity even from very brief exposures. Therefore, the effort to better evaluate children's cancer risks should not just focus on genotoxic carcinogens, but needs to include a broad range of carcinogens that have differing modes of action (Anderson *et al*., 2000). For the estrogenic agents, a variety of different mechanisms are being investigated, including inappropriate tissue stimulation, shifting the division/differentiation/apoptosis balance at critical stages in developing tissues, modulation of growth factors and hormone receptor levels, stable alteration in DNA methylation status, and other mechanisms that may affect the imprinting of cellular responsiveness to later hormonal stimulation (Gray *et al.*, 1997; Hendry *et al.*, 1997; Takefuta *et al.*, 2001; Schiffmann *et al.*, 1996).

The following summary focuses on post-natal exposures and resulting carcinogenesis. Transplacental carcinogenesis is also a well-recognized phenomena with its own set of toxicokinetic and mode of action considerations that needs to be examined from a comparative (fetal sensitivity vs. adult sensitivity) dose-response perspective.

3. ANIMAL CANCER DATA PERTAINING TO EARLY-LIFE SENSITIVITY

Cancer bioassays involving juvenile and adult rodents are summarized in Tables I and II. The literature was searched on Toxline and from review articles (Toth, 1968; Vesselinovitch, Rao, & Mihailovich, 1979; McConnell, 1992; Anderson *et al.*, 2000; Flammang *et al.*, 1997; Fujii, 1991) to identify rodent studies evaluating cancer responses in which exposure began in neonatal and preweanling animals. This search found that, in most cases, chemicals that were carcinogens in adults were also carcinogenic in juvenile animals. For example, in one review 33 of 37 chemicals demonstrated concordant results across age groups (Fujii, 1991); this concordance is especially noteworthy given that juvenile testing involved suboptimal designs: administration of only one dose level, an observation period of one year post dosing, and only 10–25 mice still on test at the one-year termination point. Another review also found a high concordance (18 of 22 chemicals) between juvenile animal and adult test results (McConnell, 1992). In this review, several cases of discordant results occurred in which perinatal exposure did not result in tumors, but these were associated with lactational exposure instead of a more defined dose regimen in juvenile animals. It should be noted that the basis for judging consistency across age groups was the overall bioassay result. As described in subsequent sections, there are a number of cases in which the specific results, that is, the location or type of tumors, differed across age groups.

Table I. Comparison of Tumor Induction: Short-Term Dosing in Juvenile and Adult Animals

Chemical[a] & Dose Route	Species/Age When Dosed	Target Site	% Tumors[b] Male/Female	Reference
B(a)P–i.p.	Mice/Day 1	Liver	81/18	Vesselinovitch *et al.*, 1975[e]
	Mice/Day 15	Liver	58/7	
	Mice/Day 42	Liver	9/0	
B(a)P–i.p.	Mice/Day 1	Lung	59/62	Vesselinovitch *et al.*, 1975
	Mice/Day 15	Lung	36/40	
	Mice/Day 42	Lung	38/17	
DBA–i.p.	Mice/Day 1	Lung	100[c]	Toth, 1968
DBA–s.c.	Mice/Day 60	Lung	6.9	
DEN/i.p.	Mice/Day 1	Liver	73/70	Vesselinovitch *et al.*, 1984
	Mice/Day 15	Liver	72/56	
	Mice/Day 42	Liver	18/2	
DEN–i.p.	Mice/Day 1	Lung	57/77	Vesselinovitch *et al.*, 1984
	Mice/Day 15	Lung	89/86	
	Mice/Day 42	Lung	78/81	
DMBA–s.c.[d] (20–30 mg/kg)	Mice/Day 1	Lung	85[c]	Pietra, Rappaport, & Shubik, 1961; Toth, 1968
	Mice/Day 60	Lung	15	
	Mice/Day 1	Lymphoma	29	
	Mice/Day 60	Lymphoma	7	
			Tumors per mouse	
DMBA–s.c.	Mice/Day 1	Lung	17/26	Walters, 1966
(1 × dose – 10 mg/kg)	Mice/Suckling	Lung	8/10	
	Mice/Adult	Lung	10/16	
DMBA–gavage (single dose, 50 mg/kg)	Rats/<2 weeks	Mammary	8 (females)	Meranze, Gruenstein, & Shimkin, 1969
	Rats/5–8 weeks	Mammary	56	
	Rats/26 weeks	Mammary	15	
DMBA–gavage (single dose, 100 mg/kg)	Rats/Day 20	Mammary	50 (females)	Russo, Wilgus, & Russo, 1979
	Rats/Days 30–55	Mammary	95	
	Rats/Days 70–180	Mammary	59	
DMBA–gavage (0.67 mg/kg/d)	Mice/adults (dosed 5x/wk for 6 weeks)	Mammary	65% (females)	Qing *et al.*, 1997
		Lymphoma	20%	
ENU–i.p.	Mice/Day 1	Liver	83/55	Vesselinovitch, Rao, & Milhailovich, 1979
	Mice/Day 15	Liver	88/51	
	Mice/Day 42	Liver	20/10	
	Mice/Day 1	Kidney	16[c]	Vesselinovitch, Rao, & Milhailovich, 1979
	Mice/Day 15	Kidney	19	
	Mice/Day 42	Kidney	9	
	Mice/Day 1	Lung	93[c]	Vesselinovitch, Rao, & Milhailovich, 1979
	Mice/Day 15	Lung	93	
	Mice/Day 42	Lung	88	
	Rats/Day 1	Nerve tissue	100/100	Naito, Naito, & Ito, 1981
	Rats/Day 7	Nerve tissue	71/90	
	Rats/Day 14	Nerve tissue	71/78	
	Rats/Day 21	Nerve tissue	46/29	
	Rats/Day 28	Nerve tissue	53/20	
	Rats/Adult	Nerve tissue	2[d]	Naito, Naito, & Ito, 1981; Odashima, 1980

(continues)

Table I. *(continued)*

Chemical[a] & Dose Route	Species/Age When Dosed	Target Site	% Tumors[b] Male/Female	Reference
			Tumor count	
3-MC–s.c.	Mice/Day 1	Lung	3.5x > adults	O'Gara & Kelly, 1963; Toth, 1968
	Mice/Day 7	Lung	10.5x > adults	
	Mice/Day 14	Lung	21x > adults	
	Mice/Day 28	Lung	3.5x > adults	
	Mice/Day 56	Lung	Adult count	
Urethan–s.c.	Mice/Day 1	Liver	90[c]	Liebelt, Liebelt, & Lane, 1964; Toth, 1968
Urethan–i.p.	Mice/Day 60	Liver	24	
Urethan–s.c.	Mice/Day 1	Lung	46	Liebelt, Liebelt, & Lane, 1964; Toth, 1968
Urethan–i.p.	Mice/Day 60	Lung	8	
Urethan–s.c.	Mice/Day 1	Leukemia	21[c]	Toth, 1968
	Mice/Day 5	Leukemia	17	
	Mice/Day 40	Leukemia	3	
X-ray (400 R)	Mice/Day 1	Liver	44 (females)	Sasaki *et al.*, 1978
	Mice/Day 35	Liver	14 (females)	

Note: Daily doses at different ages were equivalent unless otherwise noted.
[a]Abbreviations: B(a)P = benzo(a)pyrene; DBA = dibenz(a,h)anthracene; 3-MC = 3(-) methylchloanthrene; DEN = diethylnitrosamine; DMBA = dimethylbenzanthracene; ENU = ethylnitrosourea.
[b]Tumor incidence is after correction by subtracting control incidence from that seen in treated groups.
[c]Data combined across gender by study authors or gender not specified.
[d]Naito, Naito, and Ito (1981) and Odashima (1980) both refer to earlier work of Ivankovic and Druckery that adult sensitivity to neurogenic tumors from ENU is 50-fold less than in the perinatal period.
[e]Vesselinovitch *et al.* (1975) also provides data on minor sites, stomach and lymphoreticular tumors, which were similar or lower in incidence in neonatal mice compared to adults.

The high frequency of carcinogens that are active in juvenile animals demonstrated that the key question is not whether the majority of carcinogens will produce cancer when dosed, even for brief periods, in early life. The more pertinent issue for risk assessment is whether there is a potency differential across life stages and how this differential may be accounted for in assessing children's risks. To this end, the current analysis focuses on carcinogens for which positive bioassay results are available for both juvenile and adult animals. Tables I and II contain information on 16 carcinogens of varied structure and presumed cancer mechanism: (1) genotoxic agents requiring metabolic activation—nitrosamines, polycyclic aromatic hydrocarbons, safrole, and vinyl chloride; (2) genotoxicants that do not require metabolic activation—AZT, ENU, urethan, X-ray; (3) hormonal agents that may have some genotoxic activity—DES, tamoxifen; and (4) nongenotoxic organochlorine pesticides—dieldrin, DDT (see notes to Tables I and II for chemical abbreviations). As is common for juvenile animal studies, most of the study designs for these entries do not conform to standard cancer bioassay protocols in terms of number of animals on test, number of dose levels, and length of exposure and observation periods. Further, data were generally not available on time-to-tumor so it was not possible to factor latency into this assessment.

Although much of the data are suboptimal with respect to calculating cancer slope factors, they are useful on a qualitative and approximate quantitative basis to compare sensitivity differences across age groups. The ideal is to have data for different age groups within the same study, but chemicals are also included in the table if data for juvenile and adult animals came from separate studies. The tables do not represent an exhaustive summary of this type of carcinogenicity testing, but rather provide case examples for a range of chemical structures and modes of action. Given that juvenile animal cancer bioassay data are not available for most environmental chemicals, it is critical that the implications such case examples have for children's risk assessment be understood.

A key design feature is whether dosing in adults occurred for brief or chronic periods. Brief exposure in adults is more similar to the juvenile exposure protocol; this approach has helped researchers determine whether specific time frames in early life represent

Table II. Comparison of Tumor Induction: Short-Term Dosing in Juvenile Animals Versus Chronic Dosing in Adults

Chemical[a] & Dose Route	Species/Age When Dosed	Target Site	% Tumors[b] Male/Female	Reference
AZT–s.c. 100 mg/kg	Mice/Days 1–8	Lung	39 (females)	Diwan *et al.*, 1999
AZT-gav. 120 mg/kg	Mice/Adult–2 yrs	Lung	not elevated	NTP, 1999
		Vagina	21	NTP, 1999
Benzidine/diet	Mice/Preweaning	Liver	95/5	Vesselinovitch, Rao, & Milhailovich, 1979
	Mice/Weaning–90 wk	Liver	59/96	
B(a)P (54 mg/kg) (single i.p. dose)	Mice ($B6C3F_1$)/Day 15	Liver	79/0 (12-month sac)	Goldstein *et al.*, 1998; Culp *et al.*, 1998
B(a)P(2.9 mg/kg/d) (2-yr dietary exposure)	Mice ($B6C3F_1$)/Day 15	Stomach	78 (males)	Goldstein *et al.*, 1998; Culp *et al.*, 1998
DDT (gavage ~ 15 mg/kg/d)	Mice/Weeks 1–4	Liver	8% (males only)	Vesselinovitch, Rao, & Milhailovich, 1979
DDT (diet ~ 15 mg/kg/d)	Mice/Weeks 5–90	Liver	14%	
DDT (gavage, then diet)	Mice/Weeks 1–90	Liver	18%	
DEN/drinking water	Rats/Lifetime exposure beginning at 3 wks	Liver	5.5 – fold[d]	Peto *et al.*, 1984
	Rats/Beginning at 6 wks	Liver	1.9 ×	
	Rats/Beginning at 20 wks	Liver	1.0	
DES (s.c.) (~57 ug/kg/d)	Mice/Days 1–5	Uterine	14% (12-month sac)	Newbold, Bullock, & McLachlan, 1990
DES (s.c.) (~570 ug/kg/d)	Mice/Days 1–5	Uterine	50% (12 mnths) 90% (18 mnths)	
DES (diet) (~20 ug/kg/d)	Mice/Weeks 4–143	Thyroid	46/9	Greenman *et al.*, 1990
DES (diet) (~65 ug/kg/d)	Mice/Lifetime beginning at 4–6 wks	Mammary carcinoma	38/52	Gass, Coats, & Graham, 1964
Dieldrin (gavage ~0.8 mg/kg/d)	Mice/Weeks 1–4	Liver	5% (males only)	Vesselinovitch, Rao, & Milhailovich, 1979
Dieldrin (diet ~1 mg/kg/d)	Mice/Weeks 5–90	Liver	10%	
Dieldrin (gavage, then diet)	Mice/Weeks 1–90	Liver	28%	
Safrole/diet	Mice/Preweaning	Liver	32/1	Vesselinovitch, Rao, & Milhailovich, 1979
	Mice/Weaning–90 wk	Liver	8/56	
Tamoxifen gavage (1 mg/kg/d)	Rats/Neonatal (dosed on Days 2–5)	Uterine	20	Carthew *et al.*, 2000
		Vagina/cervix	9	
Tamoxifen s.c. (~2.9 mg/kg/d)	Mice/Neonatal (dosed on Days 1–5)	Uterine	50	Newbold *et al.*, 1997
Tamoxifen diet (~42 mg/kg/d)	Adult mice/2 yrs of dosing	Uterus No repro. organ tumors		Carthew *et al.*, 1996
	Adult rats/3 months dosing	Uterus	8	
Tamoxifen diet (~42 mg/kg/d)	Adult rats/11–20 months of dosing	Liver	100	Carthew *et al.*, 1995
VC–6000 ppm inhal.	Rats/Day 1 (5d/wk × 5 wk)	Angiosarc	48[c]	Maltoni *et al.*, 1981
	Rats/Day 90 (5d/wk × 52 wk)	Angiosarc	52	
VC–6000 ppm inhal.	Rats/Day 1 (5d/wk × 5 wk)	Liver	48[c]	Maltoni *et al.*, 1981
	Rats/Day 90 (5d/wk × 52 wk)	Liver	4	
VC–100 ppm inhal.	Rats/Day 60 (6-month exp.)	Angiosarc	5.3 (females)	Drew *et al.*, 1983
Newborns not tested	Rats/Day 60 (24-month exp.)	Angiosarc	44	
	Rats/Day 60 (6-month exp.)	Mammary	7.9 (females)	Drew *et al.*, 1983
	Rats/Day 60 (24-month exp.)	Mammary	9.1	
VC–100 ppm inhal.	Rats/Day 60 (6-month exp.)	Liver	24	Drew *et al.*, 1983
Newborns not tested	Rats/Day 60 (24-month exp.)	Liver	27	

Note: Daily doses at different ages were equivalent unless otherwise noted.
[a]Abbreviations: AZT = 3′azido-3′-deoxythymidine; B(a)P = benzo(a)pyrene; DEN = diethylnitrosamine; DDT = dichlorodiphenyltrichloroethane; DES = diethylstilbestrol; VC = vinyl chloride.
[b]Tumor incidence shown is after correction for reported control incidence.
[c]Data combined across gender by study authors or gender not specified.
[d]Values are fold increase in liver tumors relative to rats exposed beginning at 20 weeks.

special windows of vulnerability. Thus, where exposures in adult and juvenile animals are comparably short, one can directly compare sensitivity (on a mg/kg/d exposure basis) across age groups. However, such direct sensitivity comparisons are only part of the information needed for risk assessment of children. This is because the current practice of cancer risk assessment does not depend on short-term adult exposure studies, but rather involves chronic exposure (typically two-year duration) rodent bioassays beginning when the animals are sexually mature, young adults (five to six weeks of age). Thus it is important to compare the readout from short-term juvenile life studies to the more standard chronic-adult bioassay.

The following sections first describe data in Table I that directly compares sensitivity across age groups (short-term exposure in juvenile animals vs. short-term exposure in adults). This is followed by a description of data that bear on the early life versus standard rodent bioassay comparison (Table II). The hope is that by evaluating examples where these types of comparisons are possible, generalizations applicable to chemicals not tested in early-life studies may be possible.

3.1. Direct Sensitivity Comparisons: Brief Exposures in Early Life Versus Brief Exposure in Adults

Table I presents studies for which direct sensitivity comparisons are possible because exposure in juvenile animals and adults is similarly brief: one single injection in most cases. These comparisons are facilitated by the fact that many of the studies utilized essentially the same mg/kg dose in juvenile and adult animals. It should be noted that some studies report a given amount of chemical dosed for a range of body weights and so the mg/kg dose is a range rather than a fixed dose for a given age. Exposures were considered essentially equivalent if the dose range for juvenile and adult animals overlapped and were on average within 20% of one another. In cases where multiple dose levels were used for a given age group, groups receiving comparable doses were chosen for reporting unless otherwise noted in Tables I and II.

For the eight carcinogens where direct sensitivity comparisons are possible (B(a)P, DBA, DEN, DMBA, ENU, 3-MC, urethan, and X-ray), all show evidence of increased sensitivity in juvenile animals by at least two-fold. In addition, a wide range of tissues showed this increased sensitivity, including liver, lung, kidney, mammary tissue, hematopoeitic system, and nervous tissue. However, in any given study not all tissues were equally responsive to timing of exposure. For example, when the same i.p. dose of B(a)P was applied in neonates (Day 1) versus adults (Day 42), the liver tumor response in both male and female mice was nine-fold or more greater in the neonatally exposed mice. The liver tumor response was intermediate between neonates and adults when the mice were given this single dose at the intermediate age of 15 days. The results from this protocol for lung tumors showed smaller age differentials (1.6–3.6-fold differences) and Vesselinovitch *et al.* (1975) report that stomach and lymphoreticular tumors were not increased from juvenile exposures compared to adults.

DEN and ENU show a similar pattern with evidence for a large age differential for liver but not for lung under similar test protocols (Vesselinovitch, Rao, & Milhailovich, 1979; Vesselinovitch *et al.*, 1984). It is noteworthy that for all three of these cases (B(a)P, DEN, ENU), the adult rate of lung tumors was higher than for liver, providing less opportunity to see large sensitivity differences across age in this tissue. In contrast, Table I shows that the lung can be a site of considerable juvenile/adult sensitivity difference, as seen with DBA (14-fold), DMBA (six-fold), 3-MC (up to 21-fold based on tumor count instead of tumor incidence), and urethan (six-fold). Nerve tissue showed a large age-related susceptibility difference in tests with ENU, in which Day 1 newborns showed 100% incidence whereas subsequent ages showed a progressive decline and adults had a low response. ENU is also known as a potent transplacental carcinogen for neuronal tissue (Druckery, 1973). As shown in Table I, studies in rats with the well-recognized mammary carcinogen DMBA provide evidence that this tissue is more susceptible to carcinogenesis in the pubertal period than at more juvenile or later adult life stages (pubertal/adult differential of 1.6–3.7-fold).

Overall, Table I points out that direct sensitivity differences between juvenile animals and adults are commonly between three- and 10-fold, but with evidence that for certain carcinogens and tissues, the differential can be greater than an order of magnitude. This evidence points to discrete periods of vulnerability such that certain early life stages may contribute more to cancer risk than others. Given that the potency differences shown in Table I are across a range of chemicals and tissue types, it appears that the results may have fairly broad implications for children's risk assessment. This is further examined in the next

section wherein comparisons are made between juvenile exposures and adult-only *chronic* exposures.

3.2. Comparison Across Early-Life Exposures and Standard (Adult-Only) Bioassays

Table II contains bioassay data for both early-life exposure and adult-only chronic exposure for 10 carcinogens, in which for seven cases (benzidine, benzo(a)pyrene, DDT, DEN, dieldrin, safrole, and vinyl chloride) both exposure periods were contained within the same study and for three cases (AZT, DES, and tamoxifen) separate studies were used to enable this comparison. Regarding the former seven, in three cases (DDT, DEN, dieldrin), the design included test groups exposed both during the juvenile and adult periods. Thus, these studies enable a direct evaluation of whether the standard bioassay design would detect additional tumors and higher potency by including early-life exposure. All the other comparisons in Table II relate to groups exposed either in early life or as adults, but not both.

The early life versus adult-only-chronic exposure comparisons do not show the large tumor response differences noted above for the direct sensitivity comparisons because now the adult exposures are of chronic rather than acute duration. For eight of these carcinogens (AZT, benzidine, DDT, dieldrin, DEN, safrole, DES, and vinyl chloride), the similarity in administered dose (mg/kg/d during dosing period) across life stages facilitates a direct comparison of tumor response. With the exception of DEN, each of these examples provides evidence of similar tumor response per unit of administered dose for short-term juvenile exposure as compared to chronic adult-only exposure. For example, the tumor responses in juvenile and adult animals to AZT were in a similar range (22–39%) even though the tumor site shifted between neonatal and adult-only chronic exposure. In the case of DES, uterine tumors from short-term neonatal dosing were in lower yield (14%) than thyroid or mammary tumors (~40–50%) in adults given chronic dietary doses. However, the sacrifice of neonatally exposed mice after only 12 months in several dose groups likely curtailed the uterine tumor response. For tamoxifen, a relatively low dose in neonatal mice produced a 50% incidence of uterine tumors in contrast to a 15-fold higher chronic daily dose in adult mice, which failed to produce any reproductive tract tumors. Tamoxifen is also a reproductive tract carcinogen in neonatal rats, whereas higher chronic doses in adult rats target the liver with a 100% incidence of hepatocellular carcinoma by 11 months on test in some groups. Although there are insufficient data to fully evaluate the across age potency comparison for tamoxifen, it appears that the sensitivity of the liver in adult rats may counterbalance the reproductive tract sensitivity in early life leading to an overall potency that is not obviously different across age groups.

In the case of B(a)P, the comparison is between the 54 mg/kg single i.p. dose on day 15 of life compared to a 20-fold lower daily dose in adults for two years (Goldstein *et al.*, 1998; Culp *et al.*, 1998). Both these exposures yielded close to 80% tumor frequency, albeit at different target sites. The dissimilarity in administered dose makes a direct across age comparison difficult. However, the single B(a)P dose in juvenile mice occurred within a high susceptibility window that is at least two weeks long, as indicated by the Vesselinovitch *et al.* (1975) data (Table I). Thus, it is possible that lower doses spread out over a longer exposure period could have produced a comparable tumor response. Prorating the single i.p. dose over the two-week vulnerability window yields a daily dose of 3.9 mg/kg/d, which is similar to the daily dose given to adults for two years (2.9 mg/kg/d). Another consideration is that the animals exposed as juveniles were sacrificed at 12 months but the adults were sacrificed after 24 months of exposure. Thus, the evidence for B(a)P (Goldstein *et al.*, 1998; Culp *et al.*, 1998) generally supports the concept of equivalency of potency when comparing brief juvenile and chronic adult exposures. The vinyl chloride cancer potency comparison across age groups also supports this concept and is described further in Section 3.3.

Given that similar tumor responses can occur from juvenile-life-stage exposure as from chronic, adult-only exposure, one would expect that bioassays that combine juvenile and adult exposures in the same animals would yield higher tumor rates than in the adult-only group.

This hypothesis can be evaluated from three carcinogens in Table II for which a combined (early life plus adult) protocol was used. In the case of DDT, early-life gavage exposure yielded a slightly lower liver tumor incidence than adult-only dietary exposure, with the combination of the two time periods of exposure leading to a slightly higher incidence than the adult-only exposure (18% vs. 14%). However, only in the case of the combined exposure was the tumor response statistically significant (Vesselinovitch, Rao, & Milhailovich, 1979). Similar to this are the results with dieldrin, in which early-life exposure led to a slight increase in liver tumors,

adult-only chronic exposure led to a larger but still not significant increase, but the combination of early-life and adult exposures led to a clear and statistically significant increase in liver tumors (Vesselinovitch, Rao, & Milhailovich, 1979) (Table II). These two chlorinated pesticide examples provide further support for potency equivalence across early-life and adult exposures. In these cases, both juvenile-only and adult-only dosing protocols yielded similarly marginal responses that only became significant when both phases of exposure occurred in the same animals.

The DEN combined juvenile plus adult study (Peto *et al.*, 1984) did not have a separate early-life exposure component, but did contrast the DEN liver tumor response seen from chronic exposure beginning at three weeks versus beginning at six weeks (adults) or 20 weeks. Based on a Weibull model analysis that takes into account dose x time relationships and effects of intercurrent mortality on detecting tumors, these authors report that exposure beginning at three weeks of age was 1.9 times more effective at causing liver carcinogenesis than when exposure began at six weeks, and 5.5 times more effective than if dosing began at 20 weeks of age. Thus, early-life vulnerability to DEN makes an easily recognizable contribution to total life risk for liver tumors. The exposure time dependence for carcinogenesis was much smaller for the other major DEN target in these oral studies, the esophagus. It should be noted that in the three combined exposure case studies (DDT, dieldrin, DEN), there was a limited set of target tissues for microscopic analysis. Thus, the histopathology was inadequate to rule out the possibility that novel tumors could have been induced by including early-life exposure in the protocol.

Two other studies shown in Table II are also of interest. Data for the carcinogens benzidine and safrole indicate that adult-only chronic exposure leads to a large gender disparity in liver carcinogenesis in mice, with females more susceptible than males. However, for both chemicals, the opposite pattern is true in the case of juvenile-only exposures, with male mice more sensitive than females, and also more susceptible than when exposure is to adults only. This shifting gender pattern may not have a great impact on tumor potency calculations, which would likely be driven by the high rates of liver carcinogenesis in females exposed as adults. However, it does suggest that hormonal status, a known influence on mouse liver carcinogenesis (Moser *et al.*, 1997; Standeven, Wolf, & Goldsworthy, 1994), can be a key underlying factor that modulates age-related susceptibility differences for liver tumorigenesis.

3.3. U.S. EPA Cancer Risk Assessment for Vinyl Chloride

A key data set in Table II is the entry for vinyl chloride, specifically the juvenile rat five-week inhalation study beginning in Day 1 neonates. This was run alongside a one-year inhalation study that began when the rats were three months old (Maltoni *et al.*, 1981). The vinyl chloride data serve as the only example thus far of juvenile animal exposures being factored into a quantitative cancer risk assessment in a regulatory setting (U.S. EPA, 2000b). The analysis relied on the nearly equivalent angiosarcoma responses between juvenile-only versus adult-only exposure (Table II), which led U.S. EPA to conclude that the window of enhanced early-life susceptibility merits a separate risk calculation that is additive to later life risks. The early-life exposure was assigned the same cancer potency as the later-life exposure and the point was made that the early-life exposure was not to be time averaged (prorated) when calculating risk stemming from this period. Supportive evidence for this analysis are DNA adduct data demonstrating greater levels of vinyl chloride modification of DNA in juvenile rats than in adult rats (Laib *et al.*, 1989; Swenberg, Fedtke, & Fishbein, 1992).

It is also relevant to note that the bioassay of Drew *et al.* (1983) with vinyl chloride (Table II) also looked at the effect of timing of exposure on tumor response. While some dose groups suggested an equal effect from early-life exposures and others did not, these results are not of high relevance to the current discussion since the earliest exposure group was put on test at two months of age. Thus, this study does not report on the exposure window in animals likely to be most vulnerable to vinyl chloride and thus of most relevance to young children.

3.4. Comparison of Current Findings to the McConnell Review

A review of perinatal carcinogenesis in comparison to cancer bioassay results in adults was published by McConnell (1992). This review included animal bioassay results for 22 chemicals, many of which were included in the present analysis. Several cases reviewed by McConnell were not directly relevant to the current review in that they involved gestational and/or lactational exposure rather than administration

of defined doses directly to neonates. Overall, the McConnell review provided a useful, qualitative perspective in terms of which exposure periods gave rise to tumor increases, at which tumor sites, whether perinatal exposure was more likely to indicate a tumor response than from adult-only testing, and whether the property of genotoxicity conferred a different juvenile/adult sensitivity profile as compared to nongenotoxic chemicals. The results of the McConnell literature search and review are qualitatively similar to the current analysis in showing that perinatal exposure in conjunction with adult exposure usually increases the tumor incidence relative to adult-only exposure. It also reported that perinatal exposure only rarely identifies carcinogens that are not found using standard (adult-only) bioassay protocols. This finding was used by McConnell to conclude that routine use of perinatal exposures in cancer bioassay testing is not necessary for the identification of cancer hazard, but that such testing may be warranted to better quantify cancer potency for agents to which early-life exposures are likely. Finally, the McConnell review found that the genotoxicity of a chemical did not appear to alter the relationship between perinatal and adult sensitivity. The current analysis is in general agreement with these findings and, based on the quantitative trends noted above, proposes a method to account for early-life carcinogen sensitivity in cancer risk assessments (see Section 4).

4. IMPLICATIONS OF THE JUVENILE/ADULT COMPARATIVE CANCER BIOASSAY DATABASE

The bioassay results summarized in Tables I and II indicate that brief, early-life exposures can cause tumors in the same tissues and/or in additional tissues as that seen in adult-only exposures. Further, this can occur at daily doses during the exposure period that are comparable between short-term juvenile exposures and chronic adult-only exposures. This implies a window of heightened carcinogen susceptibility where brief exposures are just as important as long-term exposures that begin later in life. Heightened sensitivity of the juvenile period is supported by the direct sensitivity comparisons made in Section 3.1. This evidence suggests that if early-life exposures were included in standard rodent bioassays, greater tumor rates would be found. This was the case in the three liver carcinogenesis data sets reviewed in Section 3.2, which further supports the notion that risk assessment needs to give early-life exposures special consideration. The fact that this heightened early-life sensitivity appears to occur for carcinogens of various structures and cancer mechanisms (genotoxicants, estrogenic compounds, organochlorine-type promotors) provides a rationale for considering this heightened sensitivity a generalized phenomenon. However, the example of negative findings for phenobarbital in the newborn mouse model system (Flammang *et al.*, 1997; Fujii, 1991) points out that there can be cases where the assumption of heightened sensitivity in early life may not be true. Therefore, this assumption should be verified or modified as needed with actual early-life data for a specific carcinogen wherever possible.

An important implication for risk assessment is that the existing notion of simple additivity of risk across each day of exposure, with all exposure days equally important, may not be tenable. This concept has traditionally been combined with another assumption: that animal bioassays involve a lifetime of exposure so that we need a lifetime of human exposure for the animal-based cancer slope factor to be fully relevant. These assumptions have led to the practice of calculating cancer risks based on the lifetime averaged daily dose, which combines periods of high and low exposure. This can decrease cancer risk estimates for young children because they can have higher exposure per body weight to a variety of contaminated media (U.S. EPA, 2000a), yet these high exposure periods are typically averaged with periods of nonexposure or low-level exposure (older children and adults) to generate a lifetime average daily dose.

Since the standard risk assessment approach of averaging (prorating) exposures may not be adequate to describe children's risks, the following section proposes an alternative approach.

4.1. Incorporating Early-Life Sensitivity into Cancer Risk Assessment for Children

The proposed approach is an extension of the vinyl chloride example on IRIS. The first step is recognition that in many cases early-life exposures can represent a distinct risk period that should be considered separately from the remainder of the life span. Rather than average exposures across life stages, the more appropriate approach may be to separately calculate risks for each life stage and then add these risks to yield a total lifetime cancer risk. In this way, the unique exposure (U.S. EPA, 2000a) and toxicokinetic factors (Ginsberg *et al.*, 2002; Renwick, Dorne, & Walton, 2000) that are present in neonates and young children can be fully weighed

in the assessment. This will be facilitated by the types of children's physiologically-based toxicokinetic (PBTK) models that are currently under development (Pelekis, Gephart, & Lerman, 2001; Haddad, Restieri, & Krishnam, 1999; Gentry *et al.*, 2002; Ginsberg *et al.*, 2002).

The question is what slope factor to apply to early-life-stage exposures. In the vinyl chloride example on IRIS, the angiosarcoma response from dosing of juvenile rats was approximately equal to the response seen from long-term exposure beginning in adult rats (Table II). The other carcinogen examples in Section 3.2 provide additional support for an approximate equivalence of cancer potency between the short-term juvenile and long-term adult exposures if one does not prorate the dose across the entire life span. This is evident for AZT, DDT, dieldrin, safrole, and benzidine in that the daily dose administered during juvenile and adult periods was approximately equivalent, as was the tumor response. As described in Section 3.2, this is also evident for DES and B(a)P when taking into account design issues that make a direct comparison across age groups somewhat difficult. In two cases, DEN and tamoxifen, it would appear that somewhat greater potency was seen in juvenile animals compared to adults even without prorating exposures. In the case of DEN, this sensitivity increase was two- to five-fold for rat liver. For tamoxifen, the greater sensitivity of reproductive tract tissues in neonatal mice and rats may be counterbalanced by the high potency of tamoxifen to induce liver carcinoma in adult rats, although there is insufficient dose-response data to fully evaluate this.

Overall, the similar cancer potency between short-term early-life exposure and long-term adult exposure suggests that the same slope factor be applied to each developmental life stage where heightened cell proliferation or other susceptibility factors are likely. This is essentially the approach used by U.S. EPA for vinyl chloride. Since this appears to be true for a range of carcinogens, it may be prudent to use the vinyl chloride example as a more generalized approach for the numerous cases where early-life stage data are not available for a particular carcinogen.

It is important to note that linear low-dose cancer risk modeling from high-dose animal studies is a relatively crude procedure that does not take into account factors that may mitigate cancer risk at low environmental exposures. For example, there may be a greater probability that cellular detoxification systems or DNA repair will prevent genotoxicity at low dose as compared to high dose. However, there may also be cases in which the cancer potency calculated from high-dose experiments underpredicts the low-dose potency, as suggested for 1,3-butadiene and vinyl chloride due to metabolic activation systems becoming saturated under high-dose bioassay conditions (Legator, 1997; Koc *et al.*, 1999). The use of the linear low-dose approach is intended to be conservative and thus generally protective of the public. However, the degree to which such conservatism in risk modeling accounts for interindividual variability and children's risk has not been analyzed. The two cancer modeling issues (high dose to low dose; adult to child) can logically be seen as independent extrapolations with the current analysis providing a method to address the age group extrapolation. The proposed approach is amenable to evaluating children's cancer risks regardless of whether low-dose extrapolations involve linear or nonlinear modeling, or the assessment incorporates other approaches (e.g., margin of exposure). Given the increasing need to better characterize children's risks, assessors may find it important to move beyond the current default (equal sensitivity across age groups) and incorporate the juvenile animal and mechanistic evidence summarized in this article.

4.2. Summary of the Proposed Approach for Incorporating Children's Exposure and Vulnerability into Cancer Risk Assessments

In summary, the current analysis suggests the following approach for children's cancer risk assessment.

1. Do not prorate children's exposures over the entire life span, or mix them with exposures that occur at other ages. Instead, calculate exposures and effective internal concentrations (if toxicokinetic models appropriate for children are available) that are specific to children and keep them separate for the purpose of calculating child-specific cancer risk.
2. Apply the cancer slope factor from adult animal or human epidemiology studies to the children's exposure dose to calculate the cancer risk associated with the early-life period. Children's dosimetry should be adjusted by incorporating children's toxicokinetic factors (e.g., via PBTK modeling) to the extent possible.
3. Apply the same cancer slope factor to the average exposure that occurs across later life stages (older children, adults) to calculate the composite cancer risk from these age groups, as is now typically done.
4. Add the cancer risk from young children to that for the older age groups to yield a total

lifetime cancer risk. This approach was used in U.S. EPA's recent reassessment for vinyl chloride.

5. Consider whether two types of susceptibility (early life and adult) are sufficient to describe lifetime cancer risk. For example, if a carcinogen is capable of producing tumors in reproductive tissues, the pubertal period, during which these tissues undergo rapid cell proliferation, might possibly be an additional phase of susceptibility requiring separate calculation of exposure and risk (e.g., see DMBA mammary carcinogenesis example in Table I; Meranze, Gruenstein, & Shimkin, 1969; Russo, Wilgus, & Russo, 1979).

This proposed approach can yield substantially different risk estimates than the current methodology, which prorates children's exposure over the entire life span. For example, commonly used upper bound assumptions for ingestion of contaminated soil in a residential scenario are that young children (0–6 years of age, average body weight 15 kg) ingest 200 mg/d while adults (24 years of residence beyond childhood, average body weight 70 kg) ingest 100 mg/d (U.S. EPA, 1989, 2000c). No exposure to contaminated soil is assumed to occur beyond 30 years, the 90% percentile population distribution for location at a single residence. This converts to the following soil ingestion rates:

Young children: 13.3 mg/kg/d for 6 years (2,190 days)
Adults: 1.4 mg/kg/d for 24 years (8,760 days)
Remainder of lifespan—40 more years: no exposure to contaminated soil
Current method of calculating soil exposure dose:
(13.3 mg/kg/d $*$ 2190 days) + (1.4 mg/kg/d $*$ 8760 days)/25550 days = 1.62 mg/kg/d

Thus, the lifetime average soil ingestion rate from this simplistic residential scenario is eight-fold below the child-specific soil ingestion rate (1.62 mg/kg/d vs. 13.3 mg/kg/d). This converts to eight-fold less contaminant exposure and carcinogenic risk than if the proposed approach of not mixing or prorating childhood exposures with other portions of the life span is followed. In that approach, the contaminant exposure dose associated with 13.3 mg/kg/d of soil ingestion in children would be multiplied by the adult-based cancer slope factor to yield the cancer risk from childhood soil exposure. This would then be added to the cancer risk from the adult phase of exposure to yield the cumulative lifetime cancer risk.

4.3. Uncertainties

This proposed approach contains a number of uncertainties that can be addressed only by chemical-specific data that evaluates whether early-life and, perhaps, pubertal periods represent special windows of carcinogen vulnerability for that particular chemical. This is especially important for a number of common contaminants whose carcinogenicity may involve mechanisms that are different from those represented in this review (e.g., chlorinated solvents such as perchloroethylene, chloroform, methylene chloride). Application of the proposed approach to these agents represents an upper bound on risk that is inclusive of children's risk considerations; the lack of juvenile animal data precludes a more definitive approach for these agents at present.

The current analysis is semi-quantitative in that it provides a crude comparison of potency between juvenile and adult animals. This approach can be a useful starting point for a more quantitative analysis involving potency calculations for each bioassay (where sufficient data are available) and evaluating the distribution of potency results across age groups. This more quantitative level of analysis could potentially lead to refinements in the currently proposed approach.

It is also important to note that there may be cases in which juvenile animal cancer bioassays do not elicit tumors, with phenobarbital the prime example thus far (Flammang *et al.*, 1997; Fujii, 1991). This review focuses on cases in which brief carcinogen exposure in early life did elicit tumors to allow comparison of potency between juvenile and adult animals. Based on previous reviews (McConnell, 1992; Fujii, 1991), negative findings in juvenile animals with known adult carcinogens appear to be an exception rather than the rule. However, they may be indicative of an important subset that involve promotional (or other) mechanisms that are not active over the short window of exposure involved in early-life bioassays. For example, mitogenic stimulation such as from phenobarbital would generally be expected to have a smaller population of initiated cells to promote in young children than in adults (Anderson *et al.*, 2000). It is important to understand the basis for the lack of carcinogen activity in juvenile animals in cases where this can be shown to occur. This may enable better identification of chemicals for which the proposed approach would not apply.

Assessments relying on juvenile animal data contain an uncertainty regarding the rate of chemical activation and detoxification in neonates as compared to adults. A variety of toxicokinetic factors differ in

rate of development between juvenile animals and human neonates (Renwick, 1998; Renwick, Dorne, & Walton, 2000). However, the period of immature toxicokinetic function is short in human neonates relative to the period of heightened sensitivity due to rapid organ growth and cell proliferation. Many activation and detoxification functions are approaching adult levels by three to six months of age (Ginsberg *et al.*, 2002; Renwick, Dorne, & Walton, 2000), while the early life period of rapid organ growth extends beyond six months for liver and other organs (Fig. 1). This suggests that heightened periods of carcinogen vulnerability due to elevated cell proliferation rates occur over a portion of the life span in which metabolic activation systems are developing and reach fully functional (adult) levels. Thus, while the immaturity of metabolic systems may tend to decrease the formation of carcinogenic metabolites in certain cases, this is unlikely to be a significant factor for most of infancy (i.e., beyond the first three months of life).

Another uncertainty is how long a period of peak exposure during childhood is relevant to carcinogenesis. The organ growth data in Fig. 1 suggest that periods of months to years can represent heightened periods of susceptibility based on expected rates of cell proliferation. However, there may be short-term peak exposures within that susceptibility window that would tend to drive children's risks unless they are averaged out over a longer period of time. The evidence from the animal literature suggests that even very short-term exposures can result in carcinogenesis. Numerous genotoxic carcinogens in Tables I and II produced tumors from even a single dose in early life. This may involve a dose-rate function with higher doses more likely to induce tumors upon single dose exposure. Children's environmental or dietary exposures are likely to be well below the dose levels used in animal studies and bolus doses are less likely to occur. This suggests that cancer risk in children may not be driven by single exposure events to the extent shown in juvenile animals studies, with exposures best calculated based on a dose averaged over longer periods (e.g., one or more months). This averaging period may be related to the length of a particular behavior (e.g., nursing) or environmental circumstance (e.g., seasonally high influx of volatile chemicals into homes from contaminated groundwater) that involves maximal exposure. The assessment should describe the uncertainties surrounding the manner in which childhood dose is calculated.

It may appear that since this approach assumes equal potency across age groups that it makes the assumption that cancer mode of action is the same in children as in adults. However, this analysis makes no evaluation of mode of action and assumptions in this regard are not necessary for the proposed approach. There is one carcinogen, tamoxifen, for which comparative mode of action information is available in juvenile and adult animals. Tamoxifen induces tumors of the female reproductive tract from early-life exposure by what appears to be a hormonal (pro- or anti-estrogen) effect (Carthew *et al.*, 2000). Although exposed adult rats also exhibit an increase in reproductive tumors (Carthew *et al.*, 1996), tamoxifen's primary carcinogenic action in these animals is induction of liver tumors (Table I) by a mechanism involving DNA adducts (Carthew *et al.*, 1995). There is insufficient dose-response information to clarify the juvenile versus adult potency comparison, but the available data in this case do not suggest a large potency differential for these two distinct effects. The assumption of equal potency should be evaluated particularly closely in any case in which there is evidence for different modes of action across life stages.

4.4. Summary

In conclusion, the evidence for heightened periods of vulnerability in early life due to rapid cell proliferation, hormonal sensitivity, and perhaps other mechanisms, leads to a proposed approach in which early-life exposures are not prorated over the life span. The recent vinyl chloride cancer risk assessment (U.S. EPA, 2000b) acknowledges this and assumes additivity of risk across life stages based on equal potency during each key window of vulnerability. The suggestion from the current analysis is to use this approach for a wide variety of carcinogens so that the unique exposure and toxicokinetic factors in early life are not underrepresented in cancer risk assessments involving children. Chemical-specific data in juvenile animals or mechanistic data that bear on this assumption should be used wherever possible to move beyond the generalized approach on a case-by-case basis.

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