NO SIGNIFICANT RISK LEVEL (NSRL) FOR THE PROPOSITION 65 CARCINOGEN CHLOROTHALONIL

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SUMMARY OF FINDINGS

The human cancer potency of chlorothalonil was estimated and used to calculate a "No Significant Risk Level" (NSRL). The human cancer potency was estimated from dose-response data for multiple treatment-related tumor sites in male Fischer 344 rats exposed via their feed (Wilson and Killeen, 1989). An overall estimate of cancer potency associated with all treatment-related renal and forestomach tumors observed in the study was derived using a multisite statistical approach. The potency derivation takes into account differences in body size between humans and experimental animals. The human cancer potency estimate for chlorothalonil is 0.017 $(mg/kg-day)^{-1}$.

The Proposition 65 NSRL is defined in regulation as the daily intake level posing a 10^{-5} lifetime risk of cancer. The NSRL for chlorothalonil is calculated to be 41 $\mu g/day$.

Table 1. Cancer Potency and NSRL for Chlorothalonil.

Chemical	Cancer Potency $(mg/kg-day)^{-1}$	NSRL ($\mu g/day$)
Chlorothalonil	0.017	41

INTRODUCTION

This report describes the derivation of a human cancer potency estimate and NSRL for chlorothalonil (CAS No. 1897-45-6). Chlorothalonil was listed on January 1, 1989, as known to the State to cause cancer under Proposition 65 (formally known as the Safe Drinking Water and Toxic Enforcement Act of 1986; California Health and Safety Code 25249.5 *et seq.*).

Chlorothalonil is used in agriculture and horticulture as a fungicide, bactericide, and nematocide. It is also used as a preservative in wood, paints, and adhesives. Chlorothalonil is not known to occur naturally (IARC, 1999).

The studies available for cancer dose-response assessment and the derivations of the cancer potency estimate and NSRL are discussed below. A detailed description of the methodology used is provided in the Appendix.

STUDIES SUITABLE FOR DOSE-RESPONSE ASSESSMENT

There are no human cancer epidemiology studies of chlorothalonil. Several long-term animal cancer bioassays in rats and mice employing dietary administration of chlorothalonil have been conducted:

- The National Cancer Institute (NCI) conducted 80-week cancer bioassays of chlorothalonil in Osborne-Mendel rats and B6C3F$_1$ mice of both sexes (NCI, 1978).
- Bio/dynamics Inc., conducted two-year cancer bioassays in Charles River CD-1 mice of both sexes (Wilson *et al.*, 1983; Wilson *et al.*, 1986; Wilson and Killeen, 1986; all as reported in California Department of Pesticide Regulation [CDPR], 2005).
- The International Research and Development Corporation (IRDC) conducted 27-month and 30-month cancer bioassays in male and female Fischer 344 rats, respectively (Wilson, 1985 [males]; Wilson, 1986 [females]; both as reported in CDPR, 2005), and a two-year cancer bioassay in Charles River CD-1 male mice (Wilson and Killeen, 1987, as reported in California Department of Food and Agriculture [CDFA], 1988 and CDPR, 1998).
- IRDC and Experimental Pathology Laboratories, Inc. (IRDC/EPL) conducted 23- to 26-month and 29-month cancer bioassays in male and female Fischer 344 rats, respectively (Wilson and Killeen, 1989).
- Huntingdon Life Sciences conducted 80-week cancer bioassays in Crl:CD-1® (IRC)BR mice of both sexes and two-year cancer bioassays in Crl: CD®(SD)BR rats of both sexes (Spencer-Briggs,1995a [mice]; Spencer-Briggs, 1995b [rats], as reported in CDPR, 1997a [mice] and CDPR, 1997b [rats]).

In the NCI studies in Osborne-Mendel rats, groups of 10 animals/sex/control group and 50 animals/sex/treatment group were administered 0, 253 and 506 mg/kg-day chlorothalonil via their feed for 80 weeks and observed for an additional 30 weeks (NCI, 1978). Renal adenomas and carcinomas were observed in males (0/10, 2/46, and 1/49 for control, low- and high-dose groups, respectively) and females (0/10, 0/48, and 3/50 for control, low- and high-dose groups, respectively) but there were no statistically significant increases in treatment-related tumors.

In the NCI studies in B6C3F$_1$ mice, groups of 10 animals/sex/control group and 50 animals/sex/dose group were administered 0, 384, and 768 mg/kg-day (males) and 0, 429, and 851 mg/kg-day (females) chlorothalonil via their feed for 80 weeks and observed for an additional 11-12 weeks (NCI, 1978). No statistically significant increases in the incidence of treatment-related tumors were observed.

In the Bio/dynamics Inc. studies in Charles River CD-1 mice, 60 animals/sex/group were administered 0, 127, 265, and 551 mg/kg-day chlorothalonil via their feed for two years (Wilson *et al.*, 1983; Wilson *et al.*, 1986; Wilson and Killeen, 1986, as reported in CDPR, 2005). Papillomas and carcinomas of the forestomach were observed in both sexes (0/57, 2/60, 5/53, and 2/50 in males; 0/52, 2/57, 5/54, and 5/51 in females), with statistically significant increases for mid-dose males (p < 0.05) and for mid- and high-dose females (p < 0.05 in both cases) as well as a significant overall trend in females (p < 0.05). Additionally, renal adenomas and carcinomas were observed in male mice

(combined incidence: 0/57, 6/60, 4/53, and 5/50) with significant increases in the low- and high-dose groups (p < 0.05 in both cases).

In the IRDC studies in Fischer 344 rats, 60 animals/sex/group were administered 0, 40, 80, and 175 mg/kg-day chlorothalonil via their feed for 27 months (males) and 30 months (females) (Wilson, 1985; Wilson, 1986, as reported in CDPR, 2005). Papillomas and carcinomas of the forestomach were observed in treated rats of both sexes with significant trends (males: p < 0.05; females: p < 0.001), with a statistically significant increase in high-dose females as compared to controls (p < 0.01) (see Table 2). Statistically significant increases in renal adenomas and carcinomas were also observed in male and female rats of both sexes at all dose levels except low-dose females, as compared to controls (Table 2), with significant trends for both sexes (p<0.0001).

In the IRDC study in Charles River CD-1 male mice, groups of 60 animals/group were administered 0.0, 1.86, 5.35, 23.2, and 99.7 mg/kg-day chlorothalonil via their feed for two years (Wilson and Killeen, 1987, as reported in CDFA, 1988, and CDPR, 1998). No statistically significant increases in the incidence of treatment-related tumors were observed.

In the IRDC/EPL studies in Fischer 344 rats, 55 animals/sex/group were administered 0, 2, 4, 16, and 182 mg/kg-day chlorothalonil via their feed for 99 weeks in high-dose males, 111 weeks for all other males, and 125 weeks for all females (Wilson and Killeen, 1989). Papillomas and carcinomas of the forestomach were observed in female rats with a significant trend (p<0.001) and with a statistically significant increase in high-dose females as compared to controls (p < 0.01) (see Table 3). Papillomas of the forestomach were observed in male rats with a significant trend (p<0.01) and with a statistically significant increase in high-dose males as compared to controls (p < 0.05) (Table 3). Renal adenomas and carcinomas were also observed in males and females with significant trends (p < 0.0001) and with statistically significant increases in high-dose rats of both sexes (p < 0.0001) (Table 3).

In the Huntingdon Life Sciences studies in Crl:CD-1® (IRC) BR mice, 50 animals/sex/group were administered 0.0, 2.2, 8.9, 35.5, and 143.5 mg/kg-day chlorothalonil via their feed for 80 weeks (Spencer-Briggs,1995a, as reported in CDPR, 1997a). Epithelial hyperplasia of the non-glandular forestomach and the limiting ridge was increased in male mice at all dose levels. Squamous cell papillomas of the non-glandular forestomach were observed in males (1/50, 0/50, 0/50, 2/50, and 4/50) with a significant trend (p < 0.01) and females (0/50, 0/50, 1/49, 0/50, and 5/50) with a significant trend (p < 0.0001), and with a statistically significant increase in high-dose females (p < 0.05).

In the Huntingdon Life Sciences studies in Crl:CD®(SD)BR rats, groups of 50 animals/sex/group were administered 0.0, 0.8, 3.0, 12.3, and 62.0 mg/kg-day chlorothalonil via their feed for two years (Spencer-Briggs,1995b, as cited in CDPR, 1997b). Epithelial hyperplasia and hyperkeratosis of the non-glandular forestomach were increased in both sexes at all dose levels. Squamous cell papillomas and carcinomas of the forestomach were observed in males (0/50, 0/50, 0/50, 0/50, and 3/50) with a significant trend (p < 0.01) and females (0/50, 0/50, 0/50, 2/50, and 1/50),

but no statistically significant increases in the incidence of treatment-related tumors were observed.

In consideration of the studies identified above, the most suitable carcinogenicity data for human cancer potency assessments come from the longer-term studies conducted in Fischer 344 rats by the IRDC (Wilson, 1985; Wilson, 1986, as reported in CDPR, 2005) and by IRDC/EPL (Wilson and Killeen, 1989). This is based on i) observations that rats appear to be more sensitive to the carcinogenic effects of chlorothalonil than mice; ii) the exposure durations of the IRDC and IRDC/EPL Fischer 344 rat studies were greater than the other available long-term rat studies (*i.e.*, greater than two years); and iii) chlorothalonil was a more potent carcinogen in the IRDC and IRDC/EPL Fischer 344 rat studies than in the other available long-term rat studies. The dose response data for each study are presented in Tables 2 and 3 below.

Table 2. Tumor incidence in Fischer 344 rats administered chlorothalonil via feed for 27 months (males) and 30 months (females) (Wilson, 1985; Wilson, 1986, as reported in CDPR, 2005).

Sex, strain, species	Tumor site and type	Average daily dose[a] (mg/kg-day)	Tumor incidence[b]	Statistical significance[c]
Male F344/N rats	Forestomach papilloma or carcinoma	0	0/60	p < 0.05[d]
		40	1/60	NS
		80	1/60	NS
		175	3/60	NS
	Renal tubular epithelial adenoma or carcinoma	0	0/60	p < 0.0001[d]
		40	7/60	p < 0.01
		80	7/58	p < 0.01
		175	18/60	p < 0.0001
Female F344/N rats	Forestomach papilloma or carcinoma	0	0/60	p < 0.001[d]
		40	1/60	NS
		80	2/60	NS
		175	7/60	p < 0.01
	Renal tubular epithelial adenoma or carcinoma	0	0/60	p < 0.0001[d]
		40	4/60	p = 0.059
		80	10/59	p < 0.001
		175	23/60	p < 0.0001

[a] As reported by CDPR (2005) and U.S. EPA (1999)
[b] As reported by CDPR (2005)
[c] Results of pairwise comparison using Fisher's Exact Test. NS is not significant.
[d] Exact trend test p-values.

Table 3. Tumor incidence in Fischer 344 rats administered chlorothalonil via feed for 23-26 months (males) and 29 months (females) (Wilson and Killeen, 1989).

Sex, strain, species	Tumor site and type	Average daily dose[a] (mg/kg-day)	Tumor incidence[b]	Statistical significance[c]
Male F344/N rats	Forestomach papilloma	0	0/55	p < 0.01[d]
		2	0/54	NS
		4	3/54	NS
		16	2/54	NS
		182	5/55	p < 0.05
	Renal tubular epithelial adenoma or carcinoma	0	1/55	p < 0.0001[d]
		2	1/54	NS
		4	1/54	NS
		16	4/54	NS
		182	23/55	p < 0.0001
Female F344/N rats	Forestomach papilloma or carcinoma	0	1/55	p < 0.001[d]
		2	1/54	NS
		4	2/55	NS
		16	5/53	NS
		182	9/55	p < 0.01
	Renal tubular epithelial adenoma or carcinoma	0	0/55	p < 0.0001[d]
		2	0/54	NS
		4	0/55	NS
		16	0/53	NS
		182	32/55	p < 0.0001

[a] Calculated from data reported in Wilson and Killeen (1989)
[b] As reported by Wilson and Killeen (1989)
[c] Results of pairwise comparison using Fisher's Exact Test. NS is not significant.
[d] Exact trend test p-values.

APPROACH TO DOSE-RESPONSE ANALYSIS

This section briefly reviews the genotoxicity data and other data relevant to possible mechanisms of chlorothalonil carcinogenicity for the purpose of determining the most appropriate approach to dose-response analysis.

Genotoxicity

Chlorothalonil has been tested for gene mutations, chromosomal effects, and DNA damage in a variety of assays. It was positive in some but not in others. For example, as summarized by IARC (1999) and CDPR (2005), chlorothalonil induced mutations in bacteria in *Salmonella typhimurium* TA102, in the presence of metabolic activation (*i.e.*, addition of S9), but not in *S. typhimurium* TA98, TA100, TA1535, TA1537, or TA1538, or in *Escherichia coli* WP2 *hcr*. Chlorothalonil induced mutations in the yeast *Aspergillus nidulans*, and in L5178Y $t/k^{+/-}$ mouse lymphoma cells in two of three independent experiments (IARC, 1999; CDPR, 2005). With regard to chromosomal effects, chlorothalonil induced sister chromatid exchanges (IARC, 1999; CDPR, 2005) and chromosomal aberrations (CA) (CDPR, 2005; Vigreux *et al.*, 1998) in Chinese hamster ovary (CHO) cells, but did not induce CA or micronuclei (MN) in Chinese hamster lung V79 cells or mouse BALB/c 3T3 fibroblasts. Chlorothalonil induced CA in the bone marrow of male Chinese hamsters in one study, but did not induce CA or MN in other studies in male Chinese hamsters, rats, or mice (CDPR, 2005).

Evidence that chlorothalonil induces DNA damage includes studies showing that it increases levels of the oxidized DNA product 8-hydroxy-2'-deoxyguanosine (8-OH-2dG), which is a mutagenic adduct, in rat liver *in vivo* in a dose-dependent manner (Lodovici *et al.,* 1997). Additional evidence comes from various *in vitro* and *in vivo* studies measuring DNA damage, as detected by the comet assay, in rodents and humans. *In vitro*, chlorothalonil increased DNA damage in human peripheral blood lymphocytes at doses that had no immediate effect on cell viability (Lebailly *et al.*, 1997) and in CHO cells in a dose-dependent manner at doses that did not induce immediate or delayed cytotoxic effects (Vigreux *et al.*, 1998; Godard *et al.*, 1999). In a study of male farmers in which mononuclear leukocytes were evaluated before and after a single day of spraying various mixtures of pesticides, increased DNA damage was observed in farmers that sprayed mixtures containing chlorothalonil (Lebailly *et al.*, 1998). This DNA damage was observed in the absence of cytotoxicity or other effects on hematologic parameters, and was attributed by Lebailly *et al.* (1998) to chlorothalonil exposure. No DNA damage was detected by the comet assay in male Sprague-Dawley rats exposed to chlorothalonil *in vivo* (Godard *et al.*, 1999). Finally, in studies investigating whether chlorothalonil binds to DNA, a single *in vitro* study reported very low levels (1-3%) of binding of ^{14}C-chlorothalonil (radiochemical purity 96%) to mammalian DNA (Rosanoff and Siegel, 1981, as cited in CDPR, 2005), while other studies did not detect any level of covalent binding with DNA (Savides *et al.*, 1987 and Shah *et al.*, 1987, as cited in CDPR, 2005).

Chlorothalonil may be genotoxic due to its ability to induce oxidative DNA damage, to form mutagenic thiol metabolites, to bind covalently with DNA, and other mechanisms yet to be identified. 8-OH-2dG adducts, which are one manifestation of oxidative DNA damage, can lead to the formation of single point mutations as a result of G:C to T:A

transversions if not repaired before DNA replication (Shibutani *et al.,* 1991). 8-OH-2dG adducts can also result in formation of DNA strand breaks, as a result of incomplete base excision repair (Hashimoto *et al.,* 2007). DNA strand breaks may manifest as chromosomal effects (*e.g.*, CA, MN) and as DNA damage detected by the comet assay. Electrophilic thiol metabolites, such as those derived from chlorothalonil-glutathione conjugates, have the potential to react directly with DNA and induce mutations (Anders and Dekant, 1998). The low level of covalent binding to DNA observed *in vitro* (Rosanoff and Siegel, 1981, as cited in CDPR, 2005) suggests the possibility that direct binding of chlorothalonil to DNA may occur to a limited extent *in vivo.*

Cell proliferation

Chlorothalonil induced cell proliferation in the forestomach and kidneys of male and female rats in long-term bioassays (*e.g.,* Wilson, 1985 and Wilson, 1986, as reported in CDPR, 2005; Wilson and Killeen, 1989; Wilkinson and Killeen, 1996). Chlorothalonil has also been shown in shorter-term studies to induce sustained cell proliferation in rat forestomach and kidneys (U.S. EPA, 1999; Wilkinson and Killeen, 1996). For example, in 90-day dietary studies in male F344 rats, increased BrdU labeling was observed in the kidneys at day 7, day 28, and day 91 in rats receiving 175 mg/kg/day chlorothalonil (U.S. EPA, 1999). Similarly, in 28-day dietary studies in male rats, increased proliferating cell nuclear antigen (PCNA) staining of proximal convoluted tubule epithelial cells and increased BrdU labeling of the forestomach were observed at day 7, day 14, day 21, and day 28 in rats receiving 175 mg/kg/day chlorothalonil (U.S. EPA, 1999).

There may be multiple mechanisms by which chlorothalonil induces cell proliferation. One proposed mechanism involves the induction of cytotoxicity, accompanied by regenerative hyperplasia (a type of cell proliferation). Another involves activation of the erythroblastic leukemia viral (ErbB-2) oncogene tyrosine kinase signal transduction pathway, which is independent of cytotoxicity. Regarding the first proposed mechanism, chlorothalonil-derived thiols have been shown to inhibit mitochondrial respiration, based on studies conducted with rat kidney subcellular fractions (Wilkinson and Killeen, 1996). Inhibition of mitochondrial respiration results in decreased formation of adenosine triphosphate (ATP), increased oxidative stress, and ultimately, cell death (Anders and Dekant, 1998). Wilkinson and Killeen (1996) proposed that cytotoxicity induced by chlorothalonil-derived thiols in the kidney leads to compensatory cell proliferation and hyperplasia that, if sustained, eventually results in tumor formation. Support for the second proposed mechanism comes from studies in LNCaP cells, a human prostate cancer cell line, in which chlorothalonil treatment increased ErbB-2 tyrosine kinase activity, mitogen-activated protein kinase (MAPK) phosphorylation, and cell proliferation (Tessier and Matsumura, 2001).

Histone protein binding

Chlorothalonil binds to cellular proteins, including histones in the nucleus. As reviewed by CDPR (2005), incubation of ^{14}C-chlorothalonil with rat liver histones *in vitro* resulted in a significant degree of binding (*i.e.*, >50% of total radioactivity). There are a number of adverse effects that might result from the binding of chlorothalonil to histone proteins which could be involved in the chemical's carcinogenicity. These possibilities include

damage to key histone proteins involved in DNA replication and transcription processes, alteration of DNA structure (*e.g.*, folding and packaging), DNA strand breaks, and alterations in global gene methylation level with resultant changes in gene expression patterns (Baccarelli and Bollati, 2009).

In summary, multiple mechanisms are likely to be involved in chlorothalonil's carcinogenicity, including one or more involving genotoxicity. Therefore the default approach using a linearized multistage model is applied to derive a cancer potency estimate for each treatment-related tumor site observed in a given experiment. The default procedures are outlined in Title 27, California Code of Regulations, section 25703. A description of the methodology used is given in the Appendix.

DOSE-RESPONSE ASSESSMENT

Animal and human cancer potency estimates were derived for chlorothalonil by fitting the multistage model to the dose-response data from studies in male and female Fischer 344 rats conducted by the IRDC (Wilson, 1985; Wilson, 1986, as reported in CDPR, 2005) and by IRDC/EPL (Wilson and Killeen, 1989) (Tables 2 and 3).

The model fitting results in an animal cancer potency estimate, as described in the Appendix. Multiplying by the applicable interspecies scaling factor gives an estimate of human cancer potency for each treatment-related tumor site. Overall cancer potency estimates are based on the sum of potency estimates when multiple tumor types are observed within a given experiment. This calculation is performed using a Monte Carlo approach to statistically sum the potencies, as described in the Appendix. The results are summarized in Table 4 below.

The interspecies scaling factor is derived from the ratio of body weight in humans (assumed to be 70 kilograms [kg]) to the body weight of the experimental animals, as explained in the Appendix. For the Wilson (1985) study in male rats, an average body weight of 0.383 kg was calculated based on time-weighted average body weight data for control males. For the Wilson (1986) study in female rats, an average body weight of 0.240 kg was calculated based on time-weighted average body weight data reported for control females. For the Wilson and Killeen (1989) studies in male and female rats, the average body weights of 0.390 kg for males and 0.240 kg for females were calculated based on data reported for controls.

As shown in Table 4, the multisite human cancer potency derived from the Wilson and Killeen (1989) study in male rats of 0.017 $(mg/kg-day)^{-1}$ is higher than that derived from either of the other three studies (*i.e.*, Wilson (1985), Wilson (1986), and the female rat study of Wilson and Killeen (1989)). This value of 0.017 $(mg/kg-day)^{-1}$ was selected as the human cancer potency estimate for chlorothalonil.

Table 4. Cancer potency estimates for chlorothalonil based on the Wilson (1985), Wilson (1986) and Wilson and Killeen (1989) studies.

Studies	Sex, strain, species	Type of neoplasm	Animal cancer potency (mg/kg-day)$^{-1}$	Human cancer potency (mg/kg-day)$^{-1}$
Wilson (1985)	Male F344/N rats	Forestomach papilloma or carcinoma	0.000554	0.002
		Renal tubular epithelial adenoma or carcinoma	0.00274	0.010
		Multisite	0.00309	0.011
Wilson (1986)	Female F344/N rats	Forestomach papilloma or carcinoma	0.000905	0.004
		Renal tubular epithelial adenoma or carcinoma	0.00309	0.013
		Multisite	0.00357	0.015
Wilson and Killeen (1989)	Male F344/N rats	Forestomach papilloma	0.000989	0.004
		Renal tubular epithelial adenoma or carcinoma	0.00407	0.015
		Multisite	0.00468	**0.017**
	Female F344/N rats	Forestomach papilloma or carcinoma	0.00162	0.007
		Renal tubular epithelial adenoma or carcinoma	0.00115	0.005
		Multisite	0.00243	0.010

Bold indicates the value selected as the basis for the NSRL.

NO SIGNIFICANT RISK LEVEL

The NSRL for Proposition 65 is the intake associated with a lifetime cancer risk of 10^{-5}. The human cancer potency estimate of 0.017 (mg/kg-day)$^{-1}$ for chlorothalonil, based on the data from male rats in the Wilson and Killeen (1989) studies, was used to calculate the NSRL for this chemical. The value of 41 μg/day was derived as shown below.

$$\text{NSRL} = \frac{10^{-5} \times 70 \text{ kg}}{0.017 \text{ (mg/kg - day)}^{-1}} \times 1000 \text{ μg / mg} = 41 \text{ μg / day}$$

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APPENDIX: METHODOLOGY USED TO DERIVE THE NSRL FOR CHLOROTHALONIL

Procedures for the development of Proposition 65 NSRLs are described in regulation in Title 27, California Code of Regulations, Sections 25701 and 25703[1]. Consistent with these procedures, the specific methods used to derive the NSRL for chlorothalonil are outlined in this Appendix.

A.1 Cancer Potency as Derived from Animal Data

Multistage polynomial model

For regulatory purposes, the lifetime probability of dying with a tumor (p) induced by an average daily dose (d) is often assumed to be (California Department of Health Services [CDHS], 1985; U.S. Environmental Protection Agency [U.S. EPA], 2002; Anderson *et al.*, 1983):

$$p(d) = 1 - \exp[-(q_0 + q_1 d + q_2 d^2 + \cdots + q_j d^j)]$$

with constraints, $q_i \geq 0$ for all i. The q_i are parameters of the model, which are taken to be constants and are estimated from the data. With four dose groups, as is the case with the Wilson (1985) and Wilson (1986) studies of chlorothalonil (as reported in CDPR, 2005), the default linearized multistage model defaults to three stages, or four parameters, q_0, q_1, q_2, and q_3. With five dose groups, as is the case with the Wilson and Killeen (1989) studies of chlorothalonil, the default linearized multistage model defaults to four stages, or five parameters, q_0, q_1, q_2, q_3, and q_4. Due to modeling constraints associated with the Wilson and Killeen (1989) female rat forestomach tumor data, a two-parameter model was used to fit these forestomach tumor data. The parameter q_0 provides the basis for estimating the background lifetime probability of the tumor (*i.e.* $1 - \exp[-(q_0)]$). The parameter q_1 is, for small doses, the ratio of excess lifetime cancer risk to the average daily dose received. The upper 95% confidence bound on q_1, estimated by maximum likelihood techniques, is referred to here as $q_{1(UCB)}$. When the experiment duration is at least the natural lifespan of the animals, the parameter $q_{1(UCB)}$ is taken as the animal cancer potency. When dose is expressed in units of mg/kg-day, the parameters q_1 and $q_{1(UCB)}$ are given in units of $(\text{mg/kg-day})^{-1}$. Details of the estimation procedure are given in Crump (1984) and Crump *et al.* (1977).

To estimate risk at low doses, potency is multiplied by average daily dose. The risk estimate obtained is referred to by the U.S. EPA (Anderson *et al.*, 1983; U.S. EPA, 2002) as "extra risk", and is equivalent to that obtained by using the Abbott (1925) correction for background incidence.

Multisite Procedure

For carcinogens that induce tumors at multiple sites and/or with different cell types in a particular species and sex, the animal cancer potency is derived by probabilistically summing the potencies from the different sites and/or cell types. This is a way of taking

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[1] All further references are to sections of Title 27 of the California Code of Regulations.

into account the multisite carcinogenicity and provides a basis for estimating the cumulative risk of carcinogen treatment-related tumors.

The linear term (q_1) of the multistage model above is first estimated based on the dose-response data for each of the treatment-related tumor sites. Statistical distributions, rather than point estimates, are generated at each site for the linear term (q_1). The distributions of q_1 for each of the treatment-related tumor sites are then statistically summed using a Monte Carlo approach and assuming independence. The sum is created by adding the linear term for each tumor site, according to its distribution, through random sampling with 100,000 trials. The upper 95 percent confidence bound on the summed distribution is taken as the multisite animal cancer potency estimate.

Adjustments for experiments of short duration

To estimate potency in animals (q_{animal}) from experiments of duration T_e, rather than the natural life span of the animals (T), it is assumed that the lifetime incidence of cancer increases with the third power of age:

$$q_{animal} = q_{1(UCB)} \cdot (T/T_e)^3$$

Following Gold and Zeiger (1997) and the U.S. Environmental Protection Agency (U.S. EPA, 1988), the natural life span of mice and rats is assumed to be two years, so that for experiments lasting T_e weeks in these rodents:

$$q_{animal} = q_{1(UCB)} \cdot (104/T_e)^3$$

Because the duration of the Wilson (1985), Wilson (1986), and Wilson and Killeen (1989) studies were each greater than 104 weeks, a correction factor to extrapolate to 104 weeks was not required and therefore $q_{animal} = q_{1(UCB)}$.

Calculation of average daily dose

For the studies by Wilson and Killeen (1989), the average daily dose of chlorothalonil was calculated based on the body weights of the animals, the amount of chlorothalonil added to the feed, and the feed consumption rates of the animals reported by Wilson and Killeen (1989). The average daily doses in the Wilson and Killeen (1989) studies were: 0, 2, 4, 16, and 182 mg/kg-day for male rats and female rats (calculated from data reported in Wilson and Killeen, 1989).

The average daily doses of chlorothalonil in the Wilson (1985) study in male rats and in the Wilson (1986) study in female rats were reported by CDPR (2005) and U.S. EPA (1999) to be: 0, 40, 80, and 175 mg/kg-day.

A.2   Interspecies Scaling

Once a potency value is estimated in animals following the techniques described above, human potency is estimated. Under Section 25703(a)(6) as recently amended, the amount of chemical per bodyweight scaled to the three-quarters power is assumed to result in the same degree of effect across species. Under this assumption, scaling to the estimated human potency (q_{human}) can be achieved by multiplying the animal potency (q_{animal}) by the ratio of human to animal body weights (bw_h/bw_a) raised to the one-fourth power when animal potency is expressed in units $(mg/kg\text{-}day)^{-1}$:

$$q_{human} = q_{animal} \bullet (bw_h / bw_a)^{1/4}$$

In the Wilson (1985), Wilson (1986), and Wilson and Killeen (1989) studies, average body weights were calculated based on time-weighted average body weight data for control animals. Average body weight was 0.383 kg for male rats in the Wilson (1985) study, 0.240 kg for female rats in the Wilson (1986) study, 0.390 kg for male rats in the Wilson and Killeen (1989) studies, and 0.240 kg for female rats in the Wilson and Killeen (1989) studies. The default human body weight is 70 kg. An example derivation of human cancer potency using the male rat multisite animal cancer potency of 0.00468 $(mg/kg\text{-}day)^{-1}$ from the Wilson and Killeen (1989) studies is shown below:

$$q_{human} = 0.00468\ (mg/kg\text{-}day)^{-1} \bullet (70\ kg\ /0.390\ kg\ )^{1/4} = 0.017\ (mg/kg\text{-}day)^{-1}$$

A.3   Risk-Specific Intake Level Calculation

The intake level (I, in mg/day) associated with a cancer risk R, from exposure is:

$$I = \frac{R \times bw_h}{q_{human}}$$

where bw_h is the human body weight, and q_{human} is the human cancer potency estimate.

Daily intake levels associated with lifetime cancer risks above 10^{-5} exceed the NSRL for cancer under Proposition 65 (Title 27, California Code of Regulations, section 25703).

Thus for a 70 kg person, the NSRL is given by:

$$NSRL = \frac{10^{-5} \times 70\ kg}{q_{human}} \times 1000\ \mu g\ /\ mg$$

APPENDIX REFERENCES

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