

1 **A Systematic Review of Carcinogenic Outcomes and Potential Mechanisms from Exposure to 2,4-D and**
2 **MCPA in the Environment**

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24 **ABSTRACT**

25 Chlorophenoxy compounds, particularly 2,4-dichlorophenoxyacetic acid (2,4-D) and 4-chloro-2-
26 methylphenoxy)acetic acid (MCPA), are amongst the most widely used herbicides in the United States
27 for both agricultural and residential applications. Epidemiologic studies suggest that exposure to 2,4-D
28 and MCPA may be associated with increased risk non-Hodgkins lymphoma (NHL), Hodgkin's disease
29 (HD), leukemia, and soft tissue sarcoma (STS). Toxicological studies in rodents show no evidence of
30 carcinogenicity, and regulatory agencies worldwide consider chlorophenoxyes as not likely to be
31 carcinogenic or unclassifiable as to carcinogenicity. This systematic review assembles the available data
32 to evaluate epidemiologic, toxicological, pharmacokinetic, exposure and biomonitoring studies with
33 respect to key cellular events noted in disease etiology and how those relate to hypothesized modes of
34 action for these constituents to determine the plausibility of an association between environmentally-
35 relevant concentrations of 2,4-D and MCPA and lymphohematopoietic cancers. The combined evidence
36 does not support a genotoxic mode of action. Although plausible hypotheses for other carcinogenic
37 modes of action exist, a comparison of biomonitoring data to oral equivalent doses calculated from
38 bioassay data show that environmental exposures are not sufficient to support a causal relationship.
39 Genetic polymorphisms exist that are known to increase the risk of developing NHL. The potential
40 interaction between these polymorphisms and exposures to chlorophenoxy compounds, particularly in
41 occupational settings, is largely unknown.

42	1.0	Introduction	5
43	1.2	Integrated Evaluation.....	10
44	2.0	Epidemiologic Studies.....	12
45	2.1	Case-Control Studies.....	15
46	2.1.1	NHL.....	15
47	2.1.2	STS.....	21
48	2.1.3	HD.....	24
49	2.1.4	Leukemia	25
50	2.2	Cohort Studies.....	26
51	2.3	Summary of Epidemiologic Studies.....	30
52	3.0	Molecular Events in Disease Progression	32
53	3.1	Direct DNA Interaction and Repair	34
54	3.2	Non-Genotoxic Mode of Action	37
55	3.3	Summary of NHL Studies.....	38
56	4.0	Absorption, Distribution, Metabolism, Elimination (ADME)	40
57	4.1	Pharmacokinetics in Animals and Humans.....	41
58	4.1.1	Absorption.....	41
59	4.1.2	Distribution	42
60	4.1.3	Metabolism	43
61	4.1.4	Elimination	44
62	4.2	Pharmacodynamics.....	46
63	4.3	Summary of Pharmacokinetic and Pharmacodynamic Studies	50
64	5.0	Toxicological Studies.....	51
65	5.1	Toxicological Studies in Animals	53
66	5.1.1	Rodent Bioassays	54
67	5.1.1.1	MCPA.....	54
68	5.1.1.2	2,4-D.....	55
69	5.1.2	Genotoxicity Based on <i>in vitro</i> or <i>in vivo</i> Animal Studies	58
70	5.1.2.1	MCPA.....	58
71	5.1.2.2	2,4-D.....	59
72	5.2	Studies in Humans and Using Human Cell Cultures.....	61
73	5.2.1	Genotoxicity	61

74	5.2.1.1	MCPA.....	62
75	5.2.1.2	2,4-D.....	63
76	5.2.2	Proliferative and Immunological Effects.....	64
77	5.2.2.3	MCPA.....	65
78	5.2.2.4	2,4-D.....	65
79	5.3	Summary of Toxicological Studies	67
80	6.0	Exposure and Biomonitoring.....	68
81	6.1	Predictors of 2,4-D and MCPA Exposure in Occupational Settings and Observed Urinary Levels	
82		69	
83	6.2	Exposure Pathways	72
84	6.3	Biomonitoring Equivalents.....	73
85	6.4	Summary of Exposure and Biomonitoring	76
86	7.0	Discussion and Conclusions	77
87		Conflict of Interest	81
88	8.0	References	81
89			

90 **1.0 Introduction**

91 The chlorophenoxy herbicides MCPA and 2,4-D are registered for a range of agricultural and residential
92 uses focused on control of post-emergent broadleaf weeds. Since 2001, 2,4-D has been the most
93 commonly used herbicide in the residential market at 8 to 11 million pounds annually and is the seventh
94 most commonly used herbicide in the agricultural market ranging from 24 to 30 million pounds annually
95 (http://www.epa.gov/pesticides/pestsales/07pestsales/usage2007_2.htm#3_5). MCPA is used less,
96 falling within the top 25 compounds used residentially and agriculturally, but is a closely related
97 compound. Phenoxy herbicides act by simulating the action of natural hormones to produce
98 uncoordinated plant growth. Their action is selective as they are toxic to dicotyledonous but not
99 monocotyledonous plants. The physical properties of chlorophenoxy compounds can vary greatly
100 according to formulation. For instance, as alkali salts they are highly water soluble (can be formulated as
101 aqueous solutions) whereas as simple esters they demonstrate low water solubility and are more
102 lipophilic (generally formulated as emulsifiable concentrates). The acid is the parent compound, but a
103 number of formulations in use contain the more water-soluble amine salts or the ester derivatives,
104 which are readily dissolved in an organic solvent. Figure 1 shows the general chemical structure of the
105 chlorophenoxy herbicides, together with the structures of the parent compounds MCPA and 2,4-D.

106 A series of rodent bioassays submitted to the USEPA in support of pesticide registration have found no
107 carcinogenic treatment-related effects for either MCPA (Bellet et al. 1999; 2001; USEPA 1997; 2004) or
108 2,4-D (Charles et al. 1996a; 1996b; USEPA 2005). Regulatory agencies in their evaluations of these two
109 constituents have found them unlikely to be human carcinogens (USEPA 2004) or unclassifiable as to
110 carcinogenicity (USEPA 2005; Health Canada 2009; IARC 1998) while the World Health Organization
111 (WHO) has concluded that 2,4-D and its salts and esters are not genotoxic, specifically, and the toxicity
112 of the salts and esters of 2,4-D is comparable to that of the acid (WHO 1996). However, a number of
113 epidemiologic studies have found positive associations between some measure of exposure either to

114 chlorophenoxy compounds and/or MCPA and/or 2,4-D in particular and an increased risk of some
115 lymphohematopoietic cancers, primarily Non-Hodgkins lymphoma (NHL) (Mills et al. 2005; McDuffie et
116 al. 2001; Morrison 1992), but also Hodgkin's Disease (HD), soft-tissue sarcoma (STS), and to a lesser
117 extent, leukemia, while others have found no associations (Hartge et al. 2005; De Roos et al. 2003; 2004)
118 or only in combination with other compounds or with multiple chlorophenoxy (Hohenadel et al. 2011).

119 Under the assumption that the epidemiologic studies reveal a potential association between exposure
120 and outcome, there must be a series of cellular events by which exposure to chlorophenoxy compounds
121 is causally related to these carcinogenic outcomes. The toxicological studies are equivocal. While the
122 traditional *in vivo* rodent assays are all negative for tumorigenic responses, and a number of *in vivo* and
123 *in vitro* studies of potential mutagenicity and clastogenicity are negative (Gollapudi et al. 1999;
124 Linnainmaa 1984; Elliott 2005), a number of other *in vitro* studies have shown weakly positive responses
125 for chromosomal aberrations, sister chromatid exchange [SCE], and increased micronucleus formation
126 and replicative index (Holland et al. 2002) but typically only at the highest concentrations and/or doses
127 tested exceeding renal transport mechanisms, or observed effects were transient. In addition, several
128 studies have demonstrated the ability of 2,4-D to interrupt cellular functions and communication
129 (Soloneski et al. 2007; Rubinstein et al. 1984), suggesting a potential non-genotoxic mode of action.

130 Chlorophenoxy compounds are known to induce P450 (Bacher and Gibson 1988), and to effectively bind
131 to plasma proteins. Both 2,4-D and MCPA have been shown in studies ranging from rats to dogs to
132 humans to be largely excreted as parent compounds and to a lesser extent as conjugates via urine
133 within hours of exposure (Saueroff et al. 1977; Aylward et al. 2010). There is general agreement that
134 chlorophenoxy compounds do not accumulate in tissues.

135 There have been numerous previous reviews evaluating the evidence for potential health effects
136 associated with exposures to 2,4-D in particular as summarized in Table 1. The USEPA, USEPA Science

137 Advisory Board, IARC, WHO, and Canadian government independently have conducted assessments of
138 the carcinogenicity of 2,4-D, MCPA, and/or chlorophenoxy compounds generally (USEPA 1997; 1991;
139 IARC 1986; WHO 1984; 1989; Canadian Centre for Toxicology, 1987; Health Canada 2009; PMRA 2007).
140 In 1991, the Center for Risk Analysis at the Harvard School of Public Health convened a panel of 13
141 scientists to weigh the evidence on the human carcinogenicity of 2,4-D (Ibrahim et al. 1991). The panel
142 based its findings on a review of the toxicological and epidemiologic literature up to that time on 2,4-D
143 and related phenoxy herbicides. The panel concluded that the toxicological data alone do not provide a
144 strong basis for determination of carcinogenicity of 2,4-D. However, although they were unable to
145 establish a cause-effect relationship, the panel concluded there was suggestive although inconclusive
146 evidence for an association between exposure to 2,4-D and NHL and that further study was warranted.
147 The panel further concluded there was little evidence of an association between 2,4-D use and soft-
148 tissue sarcoma or Hodgkins disease, and no evidence of an association between 2,4-D use and any other
149 form of cancer.

150 Focusing specifically on the epidemiologic studies, Johnson (1990) conducted a review of the association
151 between exposure to chlorophenoxy compounds and NHL, STS, HD, and other malignant lymphomas
152 based solely on occupational cohort studies, and determined the weight of evidence at that time did not
153 unequivocally support an association between use of chlorophenoxy and malignant lymphomas and/or
154 STS, and that the available occupational cohort studies had not yet accumulated sufficient person-years
155 of observation to date. Nonetheless, despite the lack of sufficient person-years of observation, cases of
156 lymphoma and STS were observed when none were expected, suggestive of a potential association.

157 Munro et al. (1992) published a “comprehensive, integrated review and evaluation of the scientific
158 evidence relating to the safety of the herbicide 2,4-D” and found no evidence for adverse effects across
159 a range of outcomes. Focusing specifically on cancer, the authors found that only the case-control

160 studies provided any evidence of an association between exposure to 2,4-D and NHL, specifically, and
161 that this association was not borne out by the cohort studies. Finally, in an evaluation of the *in vitro* and
162 *in vivo* data, they found no support for a mechanistic basis by which 2,4-D might lead to NHL.

163 Also in 1992, Morrison et al. conducted a review of the literature and determined there was reasonable
164 evidence suggesting that occupational exposure to phenoxy herbicides resulted in increased risk of
165 developing NHL. The authors noted several studies showing large increases in risk of STS with phenoxy
166 herbicide exposure, but acknowledged that other studies had failed to observe increased risks, and
167 evidence for an exposure-risk relationship was lacking. A number of the underlying studies, particularly
168 those showing elevated risks, included exposures to other constituents, such as dioxins.

169 In 1993, Bond and Rossbacher published a review of potential human carcinogenicity of the
170 chlorophenoxy herbicides MCPA, 2-(2-methyl-4-chlorophenoxy)propanoic acid (MCP), and 2-(2,4-
171 dichlorophenoxy)propionic acid (2,4-DP). They evaluated the epidemiologic evidence, particularly based
172 on European studies, for associations between exposure to chlorophenoxy herbicides and cancer,
173 including NHL, HD, and STS. The authors concluded that although suggestive evidence from
174 epidemiologic studies of associations between chlorophenoxy herbicides and increased risks for several
175 uncommon cancers existed, the evidence was inconsistent and far from conclusive. Further, none of the
176 evidence specifically implicated MCPA, MCP, or 2,4-D. Furthermore, the results of experimental studies
177 in laboratory animals did not support a causal association between exposure these three compounds
178 and cancer development. Similarly, Gandhi et al. (2000) developed a critical evaluation of cancer risk
179 from 2,4-D and found that there was no evidence for carcinogenicity of 2,4-D although there was some
180 suggestive evidence for NHL as an outcome, but without a plausible mode of action.

181 In 2002, Garabrant and Philbert (2002) reviewed the scientific evidence from studies in both humans
182 and animals relevant to cancer risks, neurologic disease, reproductive risks, and immunotoxicity of 2,4-D

183 and its salts and esters focusing particularly on studies conducted from 1995 through 2001. The authors
184 concluded that the available evidence from epidemiologic studies did not indicate any causal association
185 of any form of cancer with 2,4-D exposure. Further, they found no human evidence of adverse
186 reproductive outcomes related to 2,4-D. The available data from animal studies of acute, subchronic,
187 and chronic exposure to 2,4-D, its salts, and esters showed an unequivocal lack of systemic toxicity at
188 doses that did not exceed renal clearance mechanisms. They found no evidence that 2,4-D in any of its
189 forms activated or altered the immune system in animals at any dose. At doses exceeding or
190 approaching renal clearance mechanisms, approximately 50 mg/kg in rats (van Ravenzwaay et al. 2003),
191 2,4-D was observed to cause liver and kidney damage and irritated mucous membranes. Although
192 myotonia and alterations in gait and behavioral indices were observed following doses of 2,4-D that
193 again exceeded renal clearance mechanisms, alterations in the neurologic system of experimental
194 animals were not observed with the administration of doses in the microgram/kg/day range. The
195 authors found it unlikely that 2,4-D exhibited any potential health effects at doses below those required
196 to induce systemic toxicity.

197 Bus and Hammond (2007) summarized the findings of animal and human health studies primarily
198 conducted or sponsored by the Industry Task Force II on 2,4-D Research Data (2,4-D Task Force) using
199 the three forms of 2,4-D (acid, dimethylamine salts, and 2-ethylhexyl ester) and reported that chronic
200 and other toxicity responses were generally limited to high doses, well above those known to result in
201 non-linear pharmacokinetic behavior. They further reported that 2,4-D did not demonstrate
202 carcinogenicity or genotoxicity in animals, did not cause birth defects, and demonstrated low potential
203 for reproductive toxicity and neurotoxicity, based on the additional studies provided to the USEPA in
204 support of 2,4-D re-registration.

205 Despite these numerous reviews and several regulatory evaluations, questions as to the carcinogenic
206 potential of 2,4-D and related compounds persist, prompting this review.

207 **1.2 Integrated Evaluation**

208 There are a number of proposed approaches for evaluating the weight of evidence for a causal
209 association between a particular exposure and a set of outcomes all of which rely to some extent on the
210 use of Hill's Criteria as applied to the body of evidence (Weed 2005). But defining criteria weights, and
211 the specific details of how the criteria are applied requires a clear definition of what constitutes
212 "weight" and what constitutes "evidence" and how those components relate to each other without
213 appearing *ad hoc* or purely the result of professional judgment. Ideally, one could use decision analytic
214 techniques in which each individual study would receive a quantitative score across each of the clearly
215 defined criteria resulting in an "objective" evaluation, but this becomes somewhat intractable,
216 particularly in this case, given the large number of studies, the categories of studies (e.g., epidemiologic,
217 *in vivo* and *in vitro*, exposure, etc.), and the nuanced details of each study.

218 Hypothesis-based weight-of-evidence (Rhomberg et al. 2010; 2011) provides a useful framework for
219 evaluating hypotheses related to potential modes of action of chemical toxicity. Mode of action has
220 regulatory significance with respect to the model used to develop toxicity factors and dose-response
221 relationships for use in risk assessments (Carmichael et al. 2011), particularly with respect to
222 carcinogenic outcomes, but most frameworks start with the premise that there is tumor induction
223 observed in animal studies (Moore et al. 2008), which is not the case for 2,4-D or MCPA. Therefore,
224 under the assumption that the epidemiologic studies are suggestive of an association between exposure
225 to 2,4-D and/or MCPA and certain lymphohematopoietic outcomes, there is a benefit to evaluating those
226 lymphohematopoietic outcomes with respect to disease etiology to identify key cellular events involved
227 in either disease initiation or promotion to determine how those might relate to a potential mode of

228 action for chlorophenoxy compounds to exert their biological influence. This strawman approach
229 provides a framework for evaluating how much of the burden of disease might be attributable to
230 environmental factors, specifically exposure to chlorophenoxy compounds. Since 2001, the
231 International Lymphoma Epidemiology Consortium has dedicated itself to providing an open scientific
232 forum and collaborative platform across which to pool data and conduct analyses related to lymphomas,
233 particularly NHL. These investigators have made significant progress in identifying molecular pathways
234 and events leading to subclinical progression of disease (Harris 2001) that are explored here in the
235 context of chlorophenoxy exposures. Is there evidence for a relationship between exposure and
236 development of molecular events required for disease progression? And if so, how would exposure to
237 chlorophenoxy compounds in the environment contribute to those events? And finally, are exposure
238 concentrations sufficient to plausibly contribute to disease incidence? What is the evidence for
239 population-level exposures and how do those relate to concentrations at which effects have been
240 observed across the different categories of studies (e.g., *in vivo* and *in vitro* toxicological,
241 epidemiologic)?

242 Figure 2 shows that synthesizing this information to determine the potential for exposure to
243 chlorophenoxy compounds at environmentally-relevant concentrations to lead to specific carcinogenic
244 outcomes requires a critical evaluation of the intersection of environmental exposures (what are the
245 exposure concentrations in the environment and how do those relate to biologically-effective doses),
246 the evidence for particular effects from toxicological and epidemiological data, and what is known about
247 cellular events at the subclinical scale in terms of disease etiology. This allows an evaluation of
248 biological plausibility with respect to a hypothesized mode of action based on the best available
249 understanding of molecular events required for disease progression, evaluated in the context of what is
250 known about how these compounds exert their biological influence, and exposure conditions necessary
251 to achieve absorbed doses relevant to the pathways of interest.

252 The structure of the review is as follows. First, the rationale for focusing on lymphohematopoietic
253 cancers is provided in Section 2.0 by summarizing and evaluating the key epidemiologic studies that
254 have demonstrated an association between some measure of exposure to 2,4-D, MCPA and/or
255 chlorophenoxy compounds generally and lymphohematopoietic cancers. Section 3.0 takes a “top
256 down” approach by evaluating what is known about disease etiology to develop hypotheses concerning
257 potential modes of action by which exposure to 2,4-D and/or MCPA might lead to the particular health
258 outcomes identified in Section 2.0. Section 4.0 identifies, discusses, and interprets the literature and
259 data with respect to the kinetics of absorption, distribution, metabolism, and elimination in laboratory
260 studies in humans and animals (4.1), followed by a subsection on pharmacodynamics. Section 5.0
261 focuses on toxicological studies, both *in vivo* and *in vitro*, starting with animal studies (5.1) and then
262 available human studies (5.2). Section 6.0 discusses exposures in the environment based on the
263 available biomonitoring data and modeling studies in the context of the hypothesized modes of action.
264 This is followed by a synthesis of the evidence in Section 7.0. References are provided in Section 8.0.

265 **2.0 Epidemiologic Studies**

266 This section identifies the available epidemiologic studies and evaluates them with respect to a number
267 of questions to identify the specific carcinogenic outcomes of interest. The first set of questions relates
268 generally to study design, including:

- 269 • *How was exposure quantified?* A key limitation of epidemiological studies is related to the way
270 in which exposures are quantified at best and categorized at worst. Many epidemiological
271 studies rely on relatively crude measures of exposure such as basic occupational status (e.g.,
272 farmer, chlorophenoxy manufacturing) with years on the job as the primary measure of more or
273 less exposure. Other studies make an attempt to quantify pounds of active constituent
274 produced (for manufacturing facilities) or used (for sprayers, farmers, etc.). This information

275 may or may not be combined with estimates of duration (e.g., two months a year for 12 years,
276 etc.). Particularly for constituent usage, most estimates rely on questionnaires of various kinds,
277 and in some cases, only next of kin are available to answer these questions. Studies that are able
278 to use quantitative exposure information (e.g., biomarkers, etc.) allow for greater confidence in
279 any observed associations.

- 280 • *What covariates were evaluated?* It is important to evaluate potential covariates of interest that
281 might be related to disease (e.g., smoking) and certainly across the entire study population. This
282 could include other potential exposures (e.g., solvents, other chemicals), and even if these
283 aren't included directly in the evaluation, it is important to understand potential differences in
284 exposures across the study population (e.g., cases have higher solvent exposures than controls,
285 etc.). If the study is attempting to evaluate exposure across a number of constituents, then the
286 statistical treatment needs to reflect these multiple comparisons to avoid spurious associations.
- 287 • *Is latency considered?* Most cancers require a series of events to occur over time following
288 exposure; a study in which exposure and outcome are largely concurrent is less compelling than
289 a study that has thought through the latency question. Weisenburger (1992) suggests that the
290 latency period for NHL, HD, and leukemia for long term, chronic exposures is on the order of 10-
291 20 years as compared to short term, high intensity exposures for which the latency period is
292 significantly shorter, on the order of five to six years.
- 293 • *How long was the follow up period in cohort studies?* Related to latency but not exactly the
294 same is the follow up period in cohort studies. Again, particularly for chronic exposures and/or
295 outcomes that are not expected for some time following exposure, it is important to allow
296 enough follow up time.

- 297 • *How were cases and controls selected in case-control studies?* Clearly, systematic differences
298 across cases and controls will influence the analysis, particularly with respect to potential
299 exposures.
- 300 • *How were outcomes identified and categorized?* In general, epidemiological studies rely on ICD
301 classifications in use at the time of the study, but these change over time as our understanding
302 of clinically-relevant differences in disease become apparent. There is also the question of
303 grouping outcomes with respect to mode of action. For example, within lymphohematopoietic
304 outcomes, which include both lymphomas and leukemias, there are different cellular and
305 molecular origins to disease relevant to the potential mode of action of an exposure such that it
306 may not be appropriate to consider outcomes too broadly. That said, there may not be enough
307 power to detect measurable differences across histological subtypes (e.g., follicular vs. mantle
308 cell lymphoma).

309 Another set of questions concerns the analysis and results, including:

- 310 • *What is the power of the study?* A challenge in epidemiological studies, particularly case-control
311 studies with rare outcomes, is the power of the study to detect a relative risk of a certain
312 magnitude.
- 313 • *Is there a dose-response relationship with measures of exposure (e.g., job duration, years of use,
314 etc.)?* In general, there is an expectation that higher and/or longer exposure would be
315 associated with higher risk, depending on the potential mode of action of the compound. An
316 initiating
- 317 • *What statistical tests are used?* As mentioned above, in the event of multiple exposures and
318 comparisons, the statistical model used needs to account for that to avoid spurious associations.

319 Candidate studies were identified through a literature search, using PubMed, MEDLINE, and Web of
320 Science, for all epidemiologic studies related to 2,4-D, MCPA, and/or chlorophenoxy compounds and
321 lymphohematopoietic cancers. Search terms included “lymph*” and “2,4-D” or “MCPA” or
322 “chlorophenoxy” or “phenoxyacetic” and “human.” References for citations obtained this way were
323 carefully reviewed to identify additional relevant studies. Papers were categorized as to type of study
324 (e.g., case-control, cohort) and general cohort (e.g., Swedish forestry workers, Finnish chlorophenoxy
325 producers, US Agricultural Study, etc.). Results from the most recent, non-overlapping analyses were
326 the focus of this assessment.

327 Several studies in occupationally-exposed case-control and to a lesser extent cohort studies show
328 statistically significant associations (Figures 3 and 4) with a number of lymphohematopoietic outcomes
329 and these are the basis for concern with respect to potential health effects associated with exposures to
330 2,4-D and/or MCPA. Figure 3 provides an overview of the epidemiologic studies related to NHL as an
331 outcome, while results for the remaining cancers are graphically depicted in Figure 4.

332 **2.1 Case-Control Studies**

333 2.1.1 NHL

334 Table 2 provides a summary of available case control studies that have evaluated NHL as an outcome.
335 Figure 3 provides these results in a graphical format.

336 The strongest association between exposure to chlorophenoxy compounds and NHL is demonstrated
337 through a series of occupational case-control studies carried out in Sweden (Eriksson et al. 1981; 1992;
338 2008; Hardell and Sandstrom, 1979; Hardell et al. 1981; 1994; Hardell and Axelson 1982; Hardell and
339 Bengtsson 1983; Hardell and Eriksson 1999; Persson et al. 1989; 1993). These occupational studies
340 focused on individuals involved in manufacturing chlorophenoxy compounds, or professional sprayers,
341 particularly in the forestry and railroad industries (e.g., spraying noxious weeds to maintain rights-of-

342 way etc.). The primary criticisms of these studies (Bond et al. 1989) include possible inaccurate
343 diagnoses, observation and/or recall bias, lack of control for confounding variables, and poorly specified
344 exposures (exposure is typically defined as greater than one day). Consequently, it is difficult to infer
345 causality from these studies since there were numerous, largely statistically uncontrolled confounding
346 exposures, and exposure itself was poorly specified, relying largely on self-reported questionnaires, and
347 often without demonstrating dose-response relationships. For deceased cases, exposure categorization
348 relied on next-of-kin, which may be particularly unreliable. Exposure was defined as greater than *one*
349 *day* over many years.

350 These studies do not consistently demonstrate statistical significance or strength of association. For
351 example, Hardell and Eriksson (1999) conducted an analysis of a population-based case–control study in
352 northern and middle Sweden with 404 NHL cases and 741 controls overall, and 12 NHL cases with 11
353 controls for the MCPA-specific analyses. They used questionnaires supplemented by telephone
354 interviews to estimate exposure. They found a marginally statistically significant odds ratio for exposure
355 to MCPA, but only when a latency period greater than 30 years was assumed. For other time periods,
356 the association was not statistically significant. The odds ratio was less than 1.0 for exposure within 10–
357 20 years of NHL onset, indicating reduced risk. Only the univariate analyses showed an increased OR =
358 2.7 (95%CI 1.0 – 7.0); the multivariate analysis OR = 1.2 (95%CI = 0.6 – 2.0). That is, only when
359 exposures were individually modeled did the authors demonstrate statistical significance.

360 Another set of studies from the United States show more equivocal results. Hoar et al. (1986)
361 conducted a population-based, case-control study in Kansas based on telephone interviews with 200
362 white men diagnosed with NHL along with 1,005 controls. Use of chlorophenoxy herbicides
363 (predominantly 2,4-D) in 24 cases and 78 controls was associated with an OR = 2.2 (95%CI = 1.2 – 4.1).
364 Table 2 shows the results stratified by days per year use of 2,4-D, which shows that only the highest

365 exposure was statistically significant, and the lowest exposure predicts a higher OR than the next two
366 higher exposures. However, the number of cases and controls was very small when stratifying results.
367 Zahm et al. (1990) followed up with a population-based, case-control study in 66 counties in eastern
368 Nebraska. Telephone interviews were conducted with 201 white men diagnosed with NHL between July
369 1, 1983 and June 30, 1986 with 775 controls. The authors report a 50% increase in NHL among men who
370 mixed or applied 2,4-D (OR = 1.5, 95%CI 0.9 – 2.5). Reported ORs were largely unchanged when
371 controlling for use of other pesticides and use of protective equipment. In fact, those farmers who
372 reported typically using protective equipment had a higher OR (1.7, 95%CI = 0.9 – 3.1) as compared to
373 those who did not (OR = 1.2, 95%CI = 0.6 – 2.4). It does not appear that the study controlled for
374 smoking and/or other lifestyle factors. One issue to note with these studies is that the questionnaire
375 used to determine exposure asked only about herbicide usage generally, and therefore may not apply
376 specifically to 2,4-D. Statistically significant associations were also found with triazines (OR = 2.5, 95% CI
377 = 1.2 – 5.4), trifluralin (OR = 12.5, 95% CI = 1.6 – 116.1), and herbicides not otherwise named (OR = 5.8,
378 95% CI = 1.9 – 17.2).

379 Kogevinas et al. (1995) report on a large, international, nested case-control study sponsored by the
380 International Agency for Research on Cancer (IARC). Kogevinas et al. (1995) evaluated 11 soft tissue
381 sarcoma and 32 lymphoma cases occurring within an international cohort which were matched for age,
382 sex, and country of residence with 55 and 158 controls, respectively. Three industrial hygienists who
383 were blind to case-control status estimated exposures to 21 chemicals or mixtures. In this study, the
384 results for NHL were not statistically significant and showed ORs less than one (Table 2). Predicted ORs
385 did not show a dose-response relationship across exposures when expressed as referent, low, medium,
386 and high (e.g., the lowest exposure, in some cases, had the highest predicted OR, but sample sizes were
387 very small when defined this way). A strength of this study is the international scope, with cases and
388 controls based on world-wide cohorts.

389 Other studies, particularly those that evaluated multiple exposures and/or dose-response relationships,
390 do not demonstrate a convincing relationship between exposure and outcome. For example, Cantor et
391 al. (1992) report on a case (n = 622) control (n = 1245 population-based) study which evaluated
392 potential exposures across a wide range of pesticides, herbicides, and insecticides, and found
393 statistically significant positive associations with exposure to malathion, DDT, chlordane and lindane, but
394 not chlorophenoxys (largely 2,4-D) and NHL. Similarly, McDuffie et al. (2001) conducted a Canadian
395 multicenter population-based incident, case (n =517)-control (n=1506) study among men in a diversity
396 of occupations using an initial postal questionnaire followed by a telephone interview for those
397 reporting pesticide exposure of 10 h/year or more, and a 15% random sample of the remainder.
398 Adjusted odds ratios (ORs) were computed using conditional logistic regression stratified by the
399 matching variables of age and province of residence, and subsequently adjusted for statistically
400 significant medical variables (history of measles, mumps, cancer, allergy desensitization treatment, and
401 a positive history of cancer in first-degree relatives). They found that among major chemical classes of
402 herbicides, the risk of NHL was statistically significantly increased for exposure to phenoxy herbicides
403 (OR=1.38; 95% CI=1.06 – 1.81), although a detailed evaluation of individual phenoxy herbicides found
404 the highest individual OR for mecoprop (OR=2.33, 95%CI=1.58 – 3.44) rather than 2,4-D or MCPA.
405 Moreover, across the entire study, the highest ORs were found for aldrin (OR = 4.18, 95%CI = 1.48 –
406 11.96). In their final models, NHL was most highly associated with a personal history of cancer; a history
407 of cancer in first-degree relatives; and exposure to dicamba-containing herbicides, to mecoprop, and to
408 aldrin. Their final models did not include 2,4-D or MCPA.

409 Miligi et al. (2003) conducted a population-based case-control study in Italy based on 1,575 interviewed
410 cases and 1,232 controls in the nine agricultural study areas. Exposure to nitro-derivatives and
411 phenylimides among fungicides, hydrocarbon derivatives and insecticide oils among insecticides, and
412 the herbicide amides were the chemical classes observed to be associated with developing NHL. ORs for

413 the chlorophenoxy compounds are presented in Table 2 and are slightly elevated in some cases but all
414 statistically insignificant. Exposure was assigned as a probability of usage in terms of chemicals families
415 and active ingredients according to an ordinal scale (low, medium, and high) taking into account the
416 time period, crops and crop diseases, and treatment applied as well as the area. Industrial hygienists
417 reviewed questionnaire data on crop diseases, treatments carried out and historical periods, field
418 acreage, geographical location, and self-reported use of specific pesticides. The agronomists involved in
419 the pesticide exposure assessment based their judgments on personal local experience, national
420 statistics on pesticide use per year and administrative unit, available records of local pesticide suppliers,
421 records of pesticide purchases by the major farms, and on professional consultants for the different
422 crops. Miligi et al. (2005) report on several additional analyses that find a statistically significant OR = 4.4
423 (95% CI = 1.1 – 29.1) based on 9 cases and 3 controls related to 2,4-D usage without protective
424 equipment. The wide confidence interval (e.g., small *n*) makes it difficult to infer a relationship.

425 In an “integrative” study evaluating many potential pesticides and combinations of pesticides, De Roos
426 et al. (2003) report on a pooled analysis from three case-control studies conducted under the auspices
427 of the National Cancer Institute in the United States based on data from the 1980s. The authors used
428 these pooled data to examine pesticide exposures in farming as risk factors for NHL in men. The large
429 sample size (*n* = 3417) allowed analysis of 47 pesticides simultaneously, controlling for potential
430 confounding by other pesticides in the model, and adjusting the estimates based on a prespecified
431 variance to make them more stable. Reported use of several individual pesticides was associated with
432 increased NHL incidence, including organophosphate insecticides coumaphos, diazinon, and fonofos,
433 insecticides chlordane, dieldrin, and copper acetoarsenite, and herbicides atrazine, glyphosate, and
434 sodium chlorate. A subanalysis of these “potentially carcinogenic” pesticides suggested a positive trend
435 of risk with exposure to increasing numbers. Estimated ORs for 2,4-D and MCPA were both below one
436 (Table 2) and were not elevated nor significant in any combined model.

437 However, Mills et al. (2005) in a study involving 131 lymphohematopoietic cancers diagnosed in
438 California between 1988 and 2001 in United Farm Workers of America (UFW) members found a
439 statistically significant OR = 3.8 (95%CI = 1.85 – 7.81) for exposure to 2,4-D. This was the only statistically
440 significant association for NHL across all pesticides studied. However, while the authors included age,
441 sex, and length of union affiliation as covariates, there was no mention of controlling for smoking and/or
442 other risk factors that may be associated with NHL. Exposure was characterized by linking UFW job
443 histories (records kept by the Union) to records of pesticide use by county kept by the State of California
444 Pesticide Databank. Employment in a given crop in a given month/year in a given county was matched
445 to the corresponding application of several pesticides on that crop in a given month and county location.
446 These applications (in pounds of active ingredients applied) were summed and used as a proxy or
447 surrogate measure of pesticide exposure for both cases and controls for the two- to three-decade
448 period prior to diagnosis of the cancer. However, although exposure was better characterized than in
449 most epidemiologic studies, there was no verification of any individual exposure (e.g., true individual
450 exposures were completely unknown).

451 Orsi et al. (2009) conducted a hospital-based case-control study in six centers in France between 2000
452 and 2004. The cases were incident cases with a diagnosis of lymphoma aged 18–75 years. During the
453 same period, controls of the same age and sex as the cases were recruited in the same hospital, mainly
454 in the orthopaedic and rheumatological departments. Exposures to pesticides were evaluated through
455 specific interviews and case-by-case expert reviews. The authors calculated ORs and 95%CIs using
456 unconditional logistic regressions and did not find an increased OR for occupational exposure to
457 chlorophenoxy compounds as a class and NHL. Hohenadel et al. (2011) report on the results of the
458 Cross-Canada Study of Pesticides and Health, a case-control study of Canadian men 19 years of age or
459 older, conducted between 1991 and 1994 in six Canadian provinces (Alberta, British Columbia,
460 Manitoba, Ontario, Quebec, and Saskatchewan). A combination of postal and telephone interviews

461 were used to obtain data for covariates and for pesticide use. Stratifying respondents based on use of 2
462 or more chlorophenoxy herbicides showed a statistically significant OR = 1.78 (95%CI = 1.27 – 2.5).
463 However, a model with only exposure to 2,4-D resulted in an OR < 1, and in a larger evaluation of
464 combinations of pesticides, malathion consistently emerged as a statistically significant exposure while
465 2,4-D did not.

466 All the previous studies have involved occupational exposures, which may not be particularly relevant to
467 residential settings or the general public with respect to actual exposure levels in the population and
468 potential risks associated with a significant use of chlorophenoxy compounds. One study, however,
469 Hartge et al. (2005) explored the relationship between residential use of herbicides (primarily on lawns)
470 and NHL in a population case-control study across Iowa, metropolitan Detroit, Los Angeles, and Seattle
471 from the period 1998 to 2000. The authors calculated relative risks based on measured 2,4-D in carpet
472 dust (Table 2) as well as self-reported herbicide (specific chemicals not provided) use on lawns. The
473 authors did not observe a relationship between estimates of exposure and NHL.

474 Another study, Leiss and Savitz (1995) explored associations between home pesticide use and childhood
475 cancers in a study that grew out of childhood cancer and electromagnetic field exposure in
476 Colorado. Exposure data was collected through parental interviews, and dichotomized as "any use" vs
477 "no use" for each pesticide type and exposure period based on the question whether the yard around
478 the residence was "ever treated with insecticides or herbicides to control insects or weeds." No
479 associations with lymphomas, broadly defined, were found with all predicted ORs less than one.

480 2.1.2 STS

481 The bottom portion of Figure 4 provides a summary of the available STS studies.

482 Hardell and Sandstrom (1979) estimated an OR of 5.3 (95% CI = 2.4 – 11.5) for STS based 13 cases and 14
483 controls from the same Swedish case-control study as described previously for NHL. A follow-on study
484 by Eriksson et al. (1981) estimated an elevated although non-statistically significant OR of 4.2 for
485 chlorophenoxy exposures free from TCDD contamination (e.g., MCPA, 2,4-D, mecoprop and
486 dichlorprop). The New Zealand studies (Smith et al. 1984; Smith and Pearce 1986) estimated a slightly
487 elevated although non-statistically significant OR in one study (Smith et al. 1984, OR = 1.6, 95%CI = 0.8 –
488 3.2; 17 cases and 13 controls) and an OR less than one in another (Smith and Pearce 1986; OR = 0.7,
489 95%CI = 0.3 – 1.5; 6 cases and 46 controls).

490 Woods et al. (1987) in a study in western Washington state estimated an OR = 0.8 (95%CI = 0.5 – 1.2)
491 assuming predominantly chlorophenoxy exposures, and Cantor et al. (1992) estimated a non-significant
492 OR = 1.2 (95%CI = 0.9 – 1.6) based on 118 cases and 231 controls. Vineis et al. 1986 in a study in Italy
493 involving female rice weeders found a non-significant OR = 2.7 (95%CI = 0.59 – 12.37) based on 31 cases
494 and 73 controls. Exposure to chlorophenoxy compounds (likely including 2,4,5-T) was based on three
495 categories: no exposure, maybe exposed, and definitely exposed. Rice weeders were considered
496 exposed to phenoxy herbicides when they worked after 1950 and did not work exclusively in a small rice
497 allotment of their own. The “maybe” category was used particularly for people engaged in corn, wheat
498 and pasture growing after 1950.

499 Hoar et al. (1986) conducted a population-based, case-control study in Kansas based on telephone
500 interviews with 200 white men diagnosed with STS along with 1,005 controls. Estimated ORs were all
501 below one except for 11 cases (57 controls) with greater than 16 years of exposure (OR = 1.4, 95% CI =
502 0.6 – 3.1).

503 The strongest associations between chlorophenoxy compound exposure and STS was found by
504 Kogevinas et al. (1995) based on 10 cases and 30 controls, who estimated an OR = 10.3 (95% CI = 1.2 –

505 90.6). When stratifying results by predominantly MCPA/MCPP exposures, the estimated OR increased
506 to 11.27 (95%CI = 1.3 – 97.9) based on 10 cases and 29 controls, and decreased to 5.72 but still
507 statistically significant (95% CI 1.14 – 28.7) based on 9 cases and 24 controls for exposures identified as
508 predominantly 2,4-D related. Exposures to 21 chemicals or mixtures were estimated by three industrial
509 hygienists who were blind to the subject's case-control status, but a dichotomous exposure classification
510 was applied which likely included considerable misclassification (according to the authors p. 398) since
511 information on dates and quantities of production and spraying of the six pesticides was not consistently
512 available. Results are presented for four exposure categories (none, low, medium, high) and the
513 predicted ORs for the chlorophenoxy and MCPA do not follow a dose-response relationship, while
514 dose-response relationships were observed for TCDD, 2,4-D, and 3,4-5-T. However, results presented in
515 this way were not statistically significant except for the highest predicted exposure for chlorophenoxy
516 generally. The authors used a logistic model and developed results for each contaminant individually.

517 Another study, Leiss and Savitz (1995) explored associations between home pesticide use and childhood
518 cancers in a study that grew out of childhood cancer and electromagnetic field exposure in
519 Colorado. Exposure data was collected through parental interviews, and dichotomized as "any use" vs
520 "no use" for each pesticide type and exposure period based on the question whether the yard around
521 the residence was "ever treated with insecticides or herbicides to control insects or weeds." Separate
522 ORs were estimated for exposure during the last three months of pregnancy (OR = 0.8, 95%CI = 0.5 – 1.3
523 based on 10 cases and 79 controls), exposure between birth and two years of diagnosis (OR = 4.1, 95%CI
524 = 1.0 – 16.0), and exposure between two years of diagnosis and diagnosis (OR = 3.9, 95%CI = 1.7 – 9.2).

525 The cohort studies do not show an association between exposure and STS as an outcome. Only two of
526 the case control studies show statistically significantly increased ORs (Kogevinas et al. 1995; Leiss and

527 Savitz 1995). Leiss and Savitz (1995) focused on childhood STS and exposure was only specified as “yard
528 treatment.”

529 2.1.3 HD

530 The top portion of Figure 4 provides the results of the epidemiologic studies focusing on HD.

531 The Swedish studies (Persson et al. 1989; Hardell and Bengtsson 1983) show mixed results for HD as an
532 endpoint. Persson et al. (1989) estimated an OR = 3.8 (95%CI = 0.7 – 21) based on 4 cases and 6 controls
533 for HD cases with exposure to predominantly chlorophenoxy compounds broadly defined. The only
534 statistically significantly increased OR = 5.0 (95%CI = 2.4 – 10.2) based on 14 cases and 24 controls
535 (Hardell and Bengtsson 1983) was for a study for which exposure was categorized as at least one day of
536 exposure to chlorophenoxy compounds based on a self-administered questionnaire. A latency period of
537 at least five years was assumed by excluding all exposures within five years of diagnosis.

538 Hoar et al. (1986) conducted a population-based, case-control study in Kansas based on telephone
539 interviews with 173 white men diagnosed with HD with 1,007 controls. None of the estimated ORs for
540 HD were statistically significant, and was only greater than one for greater than 16 years of exposure
541 (OR = 1.2, 95% CI = 0.5, 2.6).

542 Finally, Orsi et al. (2009) found a non-significant OR = 2.5 (95%CI = 0.8 – 7.7) based on 6 cases and 14
543 controls for occupational exposure of agricultural workers to chlorophenoxy compounds as a class for
544 HD. This hospital-based case-control study obtained all cases of lymphoid neoplasms from the main
545 hospitals of the French cities of Brest, Caen, Nantes, Lille, Toulouse and Bordeaux between September
546 2000 and December 2004. Exposure was categorized first through a self-administered questionnaire and
547 followed up by 90 minute individual face-to-face interviews.

548 2.1.4 Leukemia

549 The middle portion of Figure 4 provides the results of the studies investigating leukemia as an endpoint.

550 To investigate whether exposure to carcinogens in an agricultural setting is related to an increased risk
551 of developing leukemia, Brown et al. (1990) conducted a population-based case-control interview study
552 of 578 white men with leukemia and 1245 controls living in Iowa and Minnesota. They found a slight,
553 but significant, elevation in risk for all leukemia (OR = 1.2) and chronic lymphocytic leukemia (OR = 1.4)
554 for farmers compared to nonfarmers, but there were no significant associations with leukemia for
555 exposure to specific herbicides (including 2,4-D and 2,4,5-T). However, significantly elevated risks for
556 leukemia of >2.0 were seen for exposure to specific animal insecticides including the organophosphates
557 crotoxyphos (OR 11.1), dichlorvos (OR 2.0), and famphur (OR 2.2) and the natural product pyrethrins
558 (OR 3.7) and the chlorinated hydrocarbon methoxychlor (OR 2.2). There were also smaller, but
559 significant, risks associated with exposure to nicotine (OR = 1.6) and DDT (OR = 1.3). Based on exposure
560 2,4-D alone, Brown et al. (1990) estimated an OR = 1.2 (95%CI = 0.9 – 1.6) based on 98 cases and 227
561 controls, and for MCPA, the estimated OR = 1.9 (95% CI = 0.8 – 4.3) based on 11 cases and 16 controls.

562 Another study, Leiss and Savitz (1995) explored associations between home pesticide use and childhood
563 cancers in a study that grew out of a study originally on childhood cancer and electromagnetic field
564 exposure in Colorado. Exposure data was collected through parental interviews, and dichotomized as
565 "any use" vs "no use" for each pesticide type and exposure period based on the question whether the
566 yard around the residence was "ever treated with insecticides or herbicides to control insects or weeds."
567 No associations with leukemia were found with all predicted ORs less than one.

568 Orsi et al. (2009) did not find an increased OR when evaluating leukemia broadly, but disaggregated by
569 subtype, found an increased OR = 4.1 (95%CI = 1.1 – 15) for hairy cell leukemia, specifically, based on 4
570 cases and 20 controls. The overall OR = 1.0 (95%CI = 0.4 – 2.5) based on 7 cases and 20 controls largely

571 exclusively exposed to chlorophenoxy compounds. This hospital-based case-control study obtained all
572 cases of lymphoid neoplasms from the main hospitals of the French cities of Brest, Caen, Nantes, Lille,
573 Toulouse and Bordeaux between September 2000 and December 2004. Exposure was categorized first
574 through a self-administered questionnaire and followed up by 90 minute individual face-to-face
575 interviews.

576 Van Maele-Fabry et al. (2008) conducted a meta-analysis focused on three cohort studies published
577 between 1984 and 2004 found a statistically significant odds ratio (OR) for exposure to chlorophenoxy
578 compounds and leukemia (OR = 1.60, 95 confidence interval (CI) = 1.02 – 2.52), although all three
579 underlying studies individually showed non-significant associations (Coggon et al. 1986; Lynge 1998;
580 Bueno de Muesquita et al. 1993 – Factory B only).

581 Agricultural risk factors for lymphohematopoietic cancers, including leukemia, in Hispanic farm workers
582 in California were examined in a nested case-control study embedded in a cohort of 139,000 ever
583 members of a farm worker labor union in California (Mills et al. 2005). Risk of leukemia was associated
584 with exposure to the pesticides mancozeb (OR = 2.35, 95% CI = 1.12 – 4.95) and toxaphene (OR = 2.20,
585 95%CI = 1.04 – 4.65) but not 2,4-D (OR = 1.03, 95%CI = 0.41 – 2.61).

586 **2.2 Cohort Studies**

587 The cohort studies, Table 3, by their design, evaluate all cancers simultaneously rather than focusing on
588 particular cancers as is often found in the case-control studies. Table 3 presents the results of the major
589 cohort studies and in general show few statistically significant associations except for two (Carrao et al.
590 1989; Jones et al. 2009). The Carrao et al. (1989) cohort consisted of 25,945 male farmers licensed
591 between 1970 and 1974 to buy and use pesticides without any further refinement of what pesticides
592 were used, how often, and in what quantities. They estimated a standardized incidence ratio (SIR) of 1.4
593 (95%CI = 1.0 – 1.9) across the category “all malignant lymphomas” (which considers all the

594 lymphohematopoietic cancers as a single category) and conclude that this is likely due to exposure to
595 chlorophenoxy compounds. The rationale for this is that first, because the higher incidence was only
596 found in predominantly arable areas, where, the authors argue, greater use is made of herbicides
597 (although the specific herbicides in use are not discussed and the assumption is that these herbicides
598 are largely chlorophenoxy with no justification), and second, the authors argue that the use of
599 chlorophenoxyacid products had increased in recent years, so much so that this must represent the
600 predominant exposure. It is therefore difficult to argue that this analysis shows much support for a
601 relationship between exposure to chlorophenoxy compounds and lymphoma.

602 The Jones et al. (2009) study is a systematic review and meta-analysis of studies of cohorts of workers in
603 the crop protection product manufacturing industry. Jones et al. (2009) estimated meta SMRs based on
604 20 individual studies and found a statistically significantly increased SMR for lymphoma, broadly
605 defined, and exposure to chlorophenoxy (SMR = 2.01; 95%CI = 1.38 – 2.93). Although the SMR for HD
606 was greater than one, it was not statistically significant. A limitation of this meta analysis is that the
607 underlying studies included all chlorophenoxy compounds, including 2,4,5-T, which, as acknowledged by
608 the authors, is likely to have been contaminated with dioxin.

609 There have been a series of studies exploring cancer mortality and/or incidence rates in a cohort of 2,4-
610 D manufacturing workers from the Dow Chemical Company in Midland, MI (Bond 1988; Bloemen et al.
611 1993; Burns et al. 2001; 2011). In the first of these, Bond (1988) estimated standardized mortality ratios
612 (SMRs) for 878 chemical workers potentially exposed to 2,4-D at any time between 1945 and 1983.
613 Observed mortality was compared with expected levels based on adjusted rates for United States white
614 men and for other male employees from a manufacturing location who were not exposed to 2,4-D.
615 Analyses by production area, duration of exposure, and cumulative dose showed no patterns suggestive
616 of a causal association between 2,4-D exposure and any other particular cause of death. Similarly,

617 follow-up studies have not provided evidence that exposures in manufacturing workers have led to
618 increased risks.

619 Wiklund et al. (1989) report on a cohort consisting of 20,245 subjects (99% men, 1% women) who had a
620 license for pesticide application issued between 1965 and 1976 in Sweden. Approximately 20% of
621 subjects reporting using herbicides during the 1950s; 51% for the 1960s and 68% for the 1970s. The
622 most commonly used herbicide across all three decades was MCPA. The authors found a decreased
623 relative risk across all cancers.

624 Bond and Rossbacher (1993) report on two studies based on cohorts that manufactured MCPA. The
625 first, Lynge et al. (1985) evaluated 4459 chemical workers from two of four companies that had
626 produced phenoxy herbicides in Denmark, although these workers were also engaged in the
627 manufacture of diverse chemical products including not only herbicides but dyes and pigments as well.
628 Roughly one third of them (n=940) had been assigned to phenoxy herbicide production or packaging.
629 MCPA and MCPP were the predominant phenoxy herbicides produced, followed by 2,4-D and 2,4-DP.
630 Five cases of soft tissue sarcoma were reported among the men as compared to expected (relative risk =
631 2.7; (95% confidence interval) 0.88-6.34) and no cases among the women. A slight deficit of total cancer
632 was noted among the combined group of chemical workers. The second, Coggon et al. (1986) examined
633 mortality and cancer incidence in 5784 men who had been employed in manufacturing or spraying
634 MCPA in the United Kingdom. Workers were classified according to their potential for exposure into
635 high, low or background based on their job titles. Overall mortality in the cohort was less than that
636 expected from national death rates, as was mortality from all neoplasms, heart disease, and diseases of
637 the respiratory system. Only one death from soft tissue sarcoma occurred compared with one expected.
638 Three men died from malignant lymphoma compared with nine expected.

639 Lynge (1998) conducted a follow-up cohort study of 2119 workers from Denmark employed at two
640 factories that produced phenoxy herbicides since 1947 and 1951, respectively. From 1947 to 1993 the
641 2119 workers showed a slightly lower overall cancer incidence than the Danish population (observed =
642 204; expected = 234.23; SIR=0.87; 95%CI = 0.8 - 1.0). Four soft-tissue sarcoma cases were observed
643 (expected = 2.47; SIR = 1.62; 95%CI = 0.4-4.1). There were six cases of NHL (expected = 5.07; SIR = 1.10;
644 95%CI = 0.4-2.6) and no significantly elevated risk of other cancers. A follow-up study by Coggon et al.
645 (1991) in 2239 men employed in the United Kingdom from 1963 – 1985 observed two deaths from NHL
646 with 0.87 expected, a difference that was not statistically significant. No cases of STS or HD were
647 recorded.

648 In a cohort study published in 2005, 't Mannetje et al. (2005) followed 813 phenoxy herbicide producers
649 699 sprayers from January 1, 1969 and January 1, 1973 respectively until December 31, 2000. The
650 authors calculated SMRs using national mortality rates and found a 24% non-significant excess cancer
651 mortality in phenoxy herbicide producers, with a significant excess for multiple myeloma. Associations
652 were stronger for those exposed to multiple agents including dioxin during production. Overall cancer
653 mortality was not increased for producers and sprayers mainly handling final technical products.

654 Burns et al. (2001) conducted a cohort study of male employees of The Dow Chemical Company who
655 manufactured or formulated 2,4-D any time from 1945 to the end of 1994. Their mortality experience
656 was compared with national rates and with more than 40,000 other company employees who worked at
657 the same location. There were no significantly increased SMRs for any of the causes of death analyzed.
658 When compared with the United States rates, the SMR for NHL was 1.00 (95%CI= 0.21 - 2.92).

659 Boers et al. (2009) report on a third follow-up of a retrospective cohort study involving two
660 chlorophenoxy herbicide manufacturing factories, producing mainly 2,4,5-T (factory A) and MCPA/MCPP

661 (factory B) found no statistically significant increases in lymphohematopoietic cancer deaths although
662 SMRs were greater than one.

663 Aside from agricultural and forestry uses of 2,4-D, the lawn care industry also uses 2,4-D. Zahm (1997)
664 conducted a retrospective cohort mortality study of 32,600 employees of a lawn care company and
665 found four deaths due to NHL (SMR = 1.15, 95%CI = 0.31 – 2.91). Two of the (male) applicators had
666 been employed longer than three years, and for those, the predicted SMR was 7.11 (95% CI = 1.78 –
667 28.42). Risks of NHL increased for male applicators, especially those employed for three or more years,
668 but no quantitative or semiquantitative measures of pesticide use or exposure were presented.

669 **2.3 Summary of Epidemiologic Studies**

670 Associations between exposures to chlorophenoxy compounds (including 2,4-D and MCPA) and
671 potential outcomes have generally been developed through occupational studies in manufacturing
672 facility workers and/or agricultural workers. Many of the underlying studies suffer from poor exposure
673 specification (e.g., not clear which phenoxy herbicides were actually used and/or manufactured,
674 whether there was cross-contamination from dioxin or other constituents, and actual exposures and
675 doses experienced by cases and/or cohorts); poor covariate control (e.g., smoking status); and/or
676 insufficient sample sizes, and insufficient follow-up for the cohort studies. Nonetheless, the results of
677 the reviews are equivocal, with some suggesting an association with NHL but others not, and most
678 indicating that an association with STS, HD, and/or leukemia is weak at best given the generally
679 observed lack of statistical significance and risk measures less than one. Those studies that included
680 more realistic exposures (e.g., a variety of pesticides etc.) tended to reduce the influence of 2,4-D
681 and/or MCPA than those studies considering only chlorophenoxy exposure alone. The few studies
682 available for exposures likely to be experienced by the general public and/or residential use of 2,4-D and
683 MCPA found no associations with health outcomes.

684 The way in which diseases are grouped and categorized plays an important role in epidemiological
685 studies. Sorting results by histological subtype can lead to small numbers and reduced power,
686 increasing the probability of finding a particular association simply by chance. However, there may be
687 important differences with respect to exposure in terms of disease outcome (e.g., exposure to a
688 particular causal agent leads to only one histological subtype). Many different types of groupings have
689 been used in analyzing epidemiologic data, primarily reflecting the classification system in use at the
690 time of diagnosis or cause of death, and a confounding factor is that these classifications change over
691 time. Our understanding of disease etiology is always growing and increasingly researchers are able to
692 identify key molecular and cellular transformations required for disease progression. This introduces a
693 challenge for epidemiologic studies in that it may not be appropriate to consider all histological subtypes
694 of a particular carcinogenic outcome relative to a hypothesized exposure (and by extension, mode of
695 action), or it may be possible to incorporate cellular changes into measures of exposure and/or effect in
696 epidemiologic studies (discussed in the next section). Table 4 provides a summary of the available
697 epidemiologic studies that have evaluated potential exposures and NHL outcomes by subtype, and
698 shows that a consistent relationship between exposures and outcomes defined by histological subtypes
699 does not emerge.

700 In summary, the available epidemiologic studies:

- 701 • Show inconsistent relationships between exposure to chlorophenoxy compounds generally, and
702 2,4-D and/or MCPA specifically, and lymphohematopoietic outcomes
 - 703 ○ Strongest association is for NHL based on case-control studies
 - 704 ○ No statistically significant associations for leukemia
 - 705 ○ Some evidence for STS and HD but with numerous confounding exposures, exposures
706 poorly specified, small sample sizes

- 707 ○ Cohort studies show no statistically significant associations across studies save one
- 708 • The strongest association appears to be between exposure to MCPA and NHL and only in the
- 709 agricultural or forestry professions
- 710 • Studies in lawn care professionals and in residential settings do not support an association
- 711 between exposure and outcome
- 712 • With few exceptions, there are no observable dose-response relationships across the studies
- 713 • The crudest measures of exposures tend to show the strongest associations
- 714 • Exposure characterization relies predominantly on self-reported questionnaires, in some cases
- 715 with follow-up interviews. “Exposed” typically defined as greater than one day of exposure
- 716 • Those studies that focused on single assumed exposures based on univariate analyses tended to
- 717 show the highest associations (e.g., statistical significance is typically only achieved through
- 718 univariate analyses)

719 **3.0 Molecular Events in Disease Progression**

720 This section focuses on the evidence for key events at the molecular level associated with an increased

721 risk of developing lymphohematopoietic cancers with a particular emphasis on NHL.

722 Lymphohematopoietic neoplasia are characterized by an uncontrolled proliferation or expansion of cells

723 originating from the bone marrow or lymphoid tissues that do not retain the capacity to differentiate

724 normally to form mature blood cells. In general, current evidence indicates the vast majority of

725 leukemia-inducing agents are believed to act through a mutagenic mode of action whereas the

726 lymphoma-inducing agents are hypothesized to most likely act through immunomodulation and related

727 effects including indirect DNA interaction (USEPA 2010). The acute and chronic myeloid leukemias

728 (CMLs), precursor lymphomas, acute lymphoblastic leukemias (ALL) – B lymphoblastic

729 leukemia/lymphoma and T lymphoblastic leukemia/lymphoma originate in hematopoietic stem or

730 progenitor cells while the majority of lymphomas (NHL, Hodgkin lymphoma, Burkitt lymphoma) and all
731 myelomas, as well as several rare leukemias/lymphomas (adult T-cell leukemia, prolymphocytic
732 leukemia, hairy cell leukemia) and one common (CLL) leukemia originate in mature lymphoid cells
733 (USEPA 2010; Harris et al. 2001). Figure 5 presents the generalized pathways by which the risk of
734 developing lymphoma is increased.

735 Non-Hodgkin lymphomas, in particular, represent a heterogeneous group of diseases deriving from
736 mature B cells (85% of cases) and, in a minority of cases, from T cells (Harris et al. 2001). Figure 6
737 provides a schematic of the individual steps in the progression of B-cells from a stem cell to a final
738 plasma cell. Most NHLs arise from the pre B-cell and mature naïve B-cell stages. This table also shows
739 the most common, unique genetic changes that have been associated with particular forms of
740 lymphoma, and the percent of cases in which these have been observed.

741 In early and late stages of B-cell development, genetic polymorphisms and environmental exposures
742 influence the fate of a B-cell and its chances of undergoing neoplastic transformation as shown in Figure
743 6. The majority of low-grade B-cell lymphomas (e.g., follicular lymphoma) originate in the germinal
744 center. This stage of B-cell development combines extensive DNA modification with vigorous
745 proliferation (Bende et al. 2007), thus, this is a susceptible development point with respect to
746 exogenous exposures. The body responds to such strand breaks and deletions by activating DNA repair
747 genes, many of which are not present in polymorphic individuals, conferring potential susceptibility.
748 Finally, following repair (or misrepair), there is another opportunity for endogenous and exogenous
749 agents to interrupt key cellular functions by causing or exacerbating chronic inflammation, cell
750 proliferation, and/or interfering with apoptosis. Although experimental models indicate that
751 chromosomal translocations contribute to lymphoma and occur in virtually all lymphomas, there is a
752 significant body of evidence indicating that these translocations alone are not sufficient to cause disease

753 in the absence of promoting mechanisms, indicating a multistage process is required for complete
754 disease to occur (Harris et al. 2001; Janz et al. 2003).

755 **3.1 Direct DNA Interaction and Repair**

756

757 A key hypothesized event in lymphomagenesis is unrepaired and/or misrepaired DNA strand breaks
758 (Harris et al. 2001; Hill et al. 2006), and specific associations with particular forms of NHL are shown in
759 Figure 6. For example, one of the most common chromosomal abnormalities in NHL is the
760 t(14;18)(q32;q21) translocation, which occurs in 70% to 90% of cases of follicular lymphoma, 20% to
761 30% of diffuse large B-cell lymphoma, and 5% to 10% of other less common subtypes (Chiu and Blair
762 2009; Harris et al. 2001; Hill et al. 2006; Kelly et al. 2010). Under normal conditions, lymphocytes must
763 strictly regulate growth and apoptosis to provide adequate immunologic defenses against infections
764 while not overwhelming the organism with inappropriate cell numbers. The t(14;18) translocation joins
765 the BCL-2 gene on chromosome 18 to the immunoglobulin heavy chain gene on chromosome 14,
766 leading to an inhibition of apoptosis through Bcl-2 overexpression and, consequently, prolonged survival
767 of the affected B cells. Evidence is growing that agricultural exposures are associated with significant
768 t(14;18) translocations (Roulland et al. 2004; Chiu et al. 2008; Chiu and Blair 2009). Recently, Agopian et
769 al. (2009) established a direct, molecular connection between agricultural pesticide use, t(14;18) in
770 blood, and malignant progression, verifying that expanded t(14;18)+ clones truly represent malignant
771 precursors for development of follicular lymphoma.

772 However, Garry et al. (1996) investigated the possible relationships between agricultural pesticide
773 exposure and the increased risk of NHL among farm workers in the north central United States by
774 performing G-banded chromosome analyses of peripheral blood from workers classified according to
775 primary types of pesticide exposure: herbicides (n = 20), insecticides (n = 18), fumigants (n = 23), and

776 occupationally unexposed controls (n = 33). The most commonly used herbicides in this study included
777 eradican (thiocarbamate) and 2,4-D, although all pesticide use was only qualitatively described.
778 Increased lymphoma risk and excess breaks involving band 18q21 in herbicide applicators were observed.
779 Given that 2,4-D was (qualitatively) the most common herbicide, a putative link was hypothesized.
780 However, another study (Garry et al. 2001) found no correlation between measured urinary levels of
781 2,4-D and observed chromosomal aberrations in occupationally-exposed forestry workers.

782 A study conducted by Schroeder et al. (2001) used pesticide data derived from a population-based, case-
783 control study conducted in Iowa and Minnesota between 1981 and 1983. The parent study included 622
784 cases and 1245 controls and was limited to men. Tumor blocks were retrieved for 248 of the 622 cases
785 (40%) in the parent case-control study and the presence of the t(14;18) translocation in tumor tissue
786 was determined by polymerase chain reaction. One hundred eighty-two of the 248 blocks (73%) were
787 successfully assayed and 37% (68) of these cases were t(14;18)-positive, whereas 63% (114) were
788 t(14;18)-negative. Schroeder et al. (2001) found that the t(14;18)-positive NHL cases tended to have
789 larger relative risks from agricultural exposures than t(14;18)-negative cases. Report ORs for specific
790 exposures: chlorophenoxy herbicide use (n=266 controls; n=17 t(14;18) positive cases; n= 30 t(14;18)
791 negative cases) resulted in an OR = 0.9 (95%CI = 0.5 – 1.5) for the positive t(14;18) cases, and OR = 1.1
792 (95%CI = 0.5 – 1.5) for the negative t(14;18) cases. For all statistically significant associations, the
793 number of positive t(14;18) cases exceeded the number of negative t(14;18) cases (e.g., lindane,
794 cyclodienes as a class, dieldrin, toxaphene, atrazine, and phthalimide, a fumigant). Estimated ORs were
795 less than one for exposure to chlorophenoxy compounds. Similarly, Chiu et al. (2006) found a consistent
796 relationship with respect to dieldrin, lindane, and toxaphene exposures, but no relationship with
797 chlorophenoxy compounds, suggesting that although the evidence is increasing that this particular
798 chromosomal aberration is significant with respect to NHL etiology (Agopian et al. 2009), there is little

799 support for a causal role for chlorophenoxy compounds in general and specifically 2,4-D (Garry et al.
800 2001).

801 Genetic polymorphisms in DNA repair genes have also been shown to contribute to lymphomagenesis
802 (Hill et al. 2006; Shen et al. 2007). As noted, DNA breaks and other types of DNA damage are strongly
803 implicated in lymphoma development, and there are five overlapping DNA repair pathways that are
804 typically invoked to repair such breaks: (1) nonhomologous end joining (NHEJ) genes, (2) homologous
805 recombination (HR) repair, (3) nucleotide excision repair (NER), (4) base excision repair (BER), and (5)
806 direct damage reversal. V(D)J recombination involves the deliberate introduction of doublestrand
807 breaks that reshuffle dozens of Ig building blocks, the V, D, and J segments. This process produces a
808 highly diverse repertoire of antibodies, which are induced by a wide spectrum of antigenic challenges.
809 Errors by the NHEJ genes responsible for ligating the V, D, and J segments are implicated at the sites of
810 rearrangements characteristic of NHL. In addition, two steps that follow V(D)J in B-cell maturation, class-
811 switch recombination and somatic hypermutation, also introduce DNA strand breaks. The observation of
812 NHL-associated translocations or aberrant hypermutation preferentially involving those regions suggests
813 that misrepair of DNA breaks during these events could also contribute to lymphomagenesis.

814 Hill et al. (2006) evaluated the risk of NHL in relation to 32 potential inherited variants in DNA repair
815 genes and found that NHL cases were more likely than controls to have a particular variant allele
816 common in recombination genes. Shen et al. (2007) report on the association between polymorphisms
817 in DNA repair systems and NHL to in a population-based case-control study in Australia to explore
818 potential susceptibility in exposed populations. Their study specifically implicates alkylating agents as
819 they found a statistically significant association between MGMT and subtypes of NHL. MGMT encodes
820 the DNA repair protein O6-methylguanine-DNA-methyltransferase (MGMT). This protein is unique
821 among DNA repair proteins because it acts alone to remove alkyl DNA adducts. Therefore, this

822 polymorphism may be significant with respect to exposure to alkylating agents. By contrast, Hill et al.
823 (2006) found no association in three variants of MGMT and risk of NHL in a US-based case (n = 1,172)
824 control (n = 982) study suggesting prevalence population admixture differences.

825 **3.2 Non-Genotoxic Mode of Action**

826
827 Numerous studies have explored potential associations between genetic markers related to cell cycle
828 regulation and specific subtypes of NHL (Bende et al. 2007) and have shown mixed results with respect
829 to concordance across studies. However, consistent associations have been found between B-cell NHL
830 with genetic variants in pro-inflammatory factors such as TNF and leptin and the association of viral,
831 bacterial, and other exogenous agents leading to persistent inflammation (Skibola et al. 2007). Chronic
832 inflammation, interruption of cell cycle regulation (e.g., apoptosis, or limiting apoptosis that should
833 occur), and clonal expansion of mutated cells through increased cell proliferation represent processes by
834 which exposure to chemicals could increase the risk of developing lymphoma as shown in the
835 generalized schematic in Figure 5. For example, two known risk factors for NHL, cyclosporine and
836 azathioprine, act through an immunosuppressive mode of action (Eastman 1997).

837 There is evidence of differential expression of both caspase genes and Bcl-2 family member genes
838 among the NHL subtypes, leading to inhibition of apoptosis thereby allowing mutated cells to
839 proliferate. This dysregulation of the balance between cell proliferation and programmed cell death
840 (Kelly et al. 2010) is a key mechanism implicated in lymphomagenesis. For example, somatic mutations in
841 CASP3 were found in two of 129 NHL cases (Soung et al. 2004) and somatic mutations in CASP10 in 15%
842 of 117 cases (Shin et al. 2002). Aggressive follicular lymphoma, a subset of NHL, is associated with
843 upregulation of genes involved in cell cycle control such as CCNE2 (cyclin E2), CCNA2 (cyclin A2), CDK2
844 (cyclin-dependent kinase 2) and genes reflecting increased metabolism and DNA synthesis (Bende et al.
845 2007). Bende et al. (2007) report on another study which observed markedly upregulated genes

846 including the growth factor/cytokine receptors MET (the hepatocyte growth factor receptor), FGFR3
847 (fibroblast growth factor receptor 3), LTBR (lymphotoxin b receptor) and PDGFRB (platelet-derived
848 growth factor receptor b) in 11 patients with follicular lymphoma.

849 De Roos et al. (2006) studied variation in metabolic genes in a population-based case-control study in
850 the United States. They selected several genes known to play a role in metabolizing a broad spectrum of
851 substrates, including pesticides, organochlorines, solvents, and PAHs, such as the phase I cytochrome
852 P450 enzymes (CYP1A1, CYP1B1, CYP2C9, and CYP2E1), the phase II glutathione S-transferases (GSTP1
853 and GSTM3), and epoxide hydrolase (EPHX1). Subjects who were heterozygous or homozygous for the
854 cytochrome P450 gene variant CYP1B1 V432L G allele were at slightly greater risk of NHL [OR = 1.27;
855 95% CI = 0.97-1.65]; these results were consistent across B-cell lymphoma subtypes and among both
856 Caucasians and individuals of African-American descent. The CYP2E1 1054T allele was associated with
857 decreased risk of NHL (CT and TT genotypes combined OR =0.59; 95% CI = 0.37-0.93), and this pattern
858 was observed among all histologic subtypes. A systematic comparison of risks by lymphoma subtype for
859 a broad range of risk factors in a population-based case-control study conducted by Morton et al. (2008)
860 found that immune dysfunction is of greater etiologic importance for diverse large cell B-cell lymphoma
861 and marginal zone lymphoma than for follicular lymphoma, but that there were strong common
862 etiologies across all NHL subtypes. This study evaluated numerous risk factors, including specific genetic
863 polymorphisms and lifestyle and dietary characteristics, and found that exposure to chlordane and
864 PCB180 showed a relationship between exposure and specific subtypes of NHL (chlorophenoxy
865 compounds were not evaluated).

866 **3.3 Summary of NHL Studies**

867

868 Given that chromosomal translocations are present in virtually all lymphomas, and very specific
869 translocations are increasingly being identified (Harris et al. 2001), there is strong evidence that such
870 chromosomal translocations are a required step in lymphomagenesis. As shown in Figure 5, many
871 different kinds of endogenous and exogenous agents (including chemicals in the environment) can
872 interact directly with DNA and cause chromosomal aberrations of this kind in pre-B and mature B-cells,
873 and the t(14;18) translocation so ubiquitous in NHL are found in 35% - 55% of healthy individuals (Janz
874 et al. 2003). Indeed, Harris et al. (2001) state that chromosomal aberrations are necessary but not
875 sufficient to actually cause NHL (p. 202), consequently, there must be additional events that occur to
876 lead to disease. Bakhshi et al. (1987) state that the t(14;18) translocation “may offer a proliferative
877 advantage but requires additional complementing genetic changes at later steps to achieve full
878 transformation (p. 2400),” as supported by Janz et al. (2003) who find that chromosomal translocations
879 are insufficient to cause disease. Morton et al. (2009) find a significant association between the risk of
880 NHL and germline variation in genes that regulate cell cycles, apoptosis, and lymphocyte development,
881 suggesting roles for both significant genetic predisposition as well as the importance of these non-
882 genotoxic mechanisms in disease etiology. USEPA (2010) suggests that immunomodulation and related
883 effects and indirect effects on DNA are the primary causal factors across the lymphomas. The evidence
884 suggests that multiple events are required to lead to NHL, most likely including some combination of
885 chromosomal translocation coupled with proliferation of a mutation, an interruption in cell cycle
886 regulation (e.g., failure to initiate apoptosis) or chronic inflammation. Based on observations of
887 molecular events significant to the development of NHL specifically and lymphomas generally, the
888 following modes of action can be hypothesized:

- 889 • Specific chromosomal aberrations (direct genotoxicity)
 - 890 ○ Gene-environment interaction in individuals with polymorphisms
 - 891 ■ Interruption of programmed cell death

- 892 ▪ DNA repair mechanisms
- 893 • Induction of enzymes implicated in the bioactivation of ubiquitous exogenous or endogenous
- 894 genotoxic compounds (indirect genotoxicity); oxidative stress
- 895 • Proliferation of mutations
- 896 • Immunotoxic responses

897 The evidence suggests a combination of molecular events is required in the etiology of NHL, including
898 specific chromosomal aberrations which are significantly increased in agriculturally-exposed individuals,
899 but not for chlorophenoxy exposure specifically (Garry et al. 2001; Chiu et al. 2009; Agopian et al. 2009).

900 There is evidence that promoting activity is also required as chromosomal aberrations, particularly those
901 associated with NHL, are prevalent in healthy individuals (Limpens et al. 1995; Bende et al. 2007),
902 including cell proliferation of mutations, chronic inflammation, and cell cycle interruptions (e.g., failure
903 to initiate apoptosis). There is growing evidence that germline polymorphisms contribute significantly
904 to NHL etiology which would increase susceptibility in these individuals. It is therefore hypothetically
905 possible that exposure to chlorophenoxy compounds in susceptible individuals could shift the risk curve
906 (e.g., lead to increased risk at lower exposure levels as compared to non-susceptible individuals) by a
907 non-genotoxic mode-of-action. The evidence does not support a genotoxic or mutagenic mode-of-
908 action; however, key transcription errors have been identified in more than half the U.S. population;
909 therefore, it is theoretically possible that exposure to 2,4-D/MCPA could lead to other cellular responses
910 that, in the presence of genetic polymorphisms, might lead to increased risk.

911 **4.0 Absorption, Distribution, Metabolism, Elimination (ADME)**

912 The potential for 2,4-D and/or MCPA exposures to lead to development of NHL is influenced by the
913 efficiency with which the compounds are absorbed across different exposure routes and disposition of
914 the compounds once in the body. Data from toxicological studies are interpreted in the context of

915 potential exposure route, and a consideration of how chlorophenoxy compounds are absorbed,
916 metabolized, dispersed, and eliminated once in the body. Data from laboratory studies following the
917 time course of absorbed exposures provides important information and these data are also used to
918 develop different kinds of models (e.g., physiologically-based pharmacokinetic [PBPK] and others) for
919 use in risk assessment and other assessments of environmental exposures. This section briefly describes
920 the results of laboratory studies that have generated ADME data and modeling studies that have used
921 these data.

922 To identify relevant citations, a literature search was conducted using PubMed, Medline and Web of
923 Science with the search terms "chlorophenox*" or "2,4-D" or "MCPA" and "pharmaco*" or "metabo*"
924 Further studies were identified through the reference lists of studies obtained through the literature
925 search. The search focused on primary citations in the peer-reviewed literature, although to the extent
926 that there were some unpublished studies utilized in regulatory or other reviews, these secondary
927 sources were summarized as well.

928 In general, 2,4-D and MCPA are eliminated via urine either as the unchanged parent compound (80–
929 95%) or as conjugates, with urinary half-lives on the order of one day with no evidence of oxidative
930 metabolism in humans (Saueroff et al. 1977; Kohli et al. 1974) or other mammals (Timchalk 2004). 2,4-D
931 and MCPA do not accumulate in tissues.

932 **4.1 Pharmacokinetics in Animals and Humans**

933

934 4.1.1 Absorption

935 MCPA and 2,4-D are readily absorbed and undergo significant but reversible plasma binding (Timchalk
936 2004; Khanna and Fang 1966; Gorzinski et al. 1987; Lappin et al. 2002; Bellet et al. 1999; Elo and Ylitalo
937 1979; van Ravenzwaay et al. 2003; 2004; 2005). For example, Khanna and Fang (1966) explored the

938 pharmacokinetics of ¹⁴C 2,4-D in male Wistar rats in two sets of experiments. In the first, six rats were
939 orally dosed with 1 mg of ¹⁴C 2,4-D per rat, while in the second, seven rats were administered an oral
940 dose of 80 mg of 2,4-D. For the 1 mg 2,4-D dosage, maximum radioactivity in all tissues was reached at
941 within eight hours of dosing, and started to decrease immediately. At the 80 mg dosage, peak
942 concentrations persisted until about 17 hours. The urine and the extracts of several tissues contained
943 primarily unchanged 2,4-D residue.

944 Studies show 2,4-D and MCPA are both readily absorbed via oral administration, but have revealed
945 species differences in dermal absorption (Ross et al. 2005) with rats showing approximately 20%
946 absorption and humans less than 10%. Ross et al. (2005) report on an analysis of all the available data
947 concerning dermal absorption of 2,4-D in humans based on five studies involving 34 subjects. The
948 studies provide remarkably similar results, ranging from 1.1% to 10% absorption with a mean of 5.7%.
949 Both the salt and acid forms of 2,4-D were evaluated with no appreciable difference, and applied doses
950 ranged from 1.7 to 1,100 µg/cm². USEPA and Health Canada have both used dermal absorption values
951 of approximately 10% for both 2,4-D and MCPA in conducting risk assessments associated with
952 reregistration of these compounds (Health Canada 2005; 2006; 2008a; 2008b; 2009; USEPA 2004a;
953 2004b; 2005; 77FR23135).

954 4.1.2 Distribution

955 2,4-D, and to a lesser extent MCPA, is highly bound to plasma proteins (van Ravenzwaay et al. 2003;
956 2004; Lappin et al. 2002; Bräunlich et al. 1989) and both are characterized by a low volume of
957 distribution (Bräunlich et al. 1989). Chlorophenoxy compounds bind largely to albumin (Bräunlich et al.
958 1989, Rosso et al. 1998; Roberts et al. 2011) and plasma binding is saturable at approximately 115 mg/L
959 based on 128 blood samples from 49 patients with acute MCPA poisoning (Roberts et al. 2011).

960 Saghir et al. (2006) examined steady state levels of 2,4-D following continuous dietary dosing in rats at 5
961 and 100 mg/kg-day for 28 days. At 5 mg/kg, the C_{max} blood concentration was 0.72 $\mu\text{g}/\text{ml}$, and was 64
962 $\mu\text{g}/\text{ml}$ at 100 mg/kg. At these dose levels, steady-state concentrations varied less than two-fold over a
963 24-hour period. The authors suggest that the mechanism of the observed non-dose-proportional
964 increase in plasma 2,4-D concentration is likely due to high-dose-dependent saturation of the renal
965 active anion transport clearance mechanism (the same mechanism for renal clearance of 2,4-D in
966 humans).

967 Elo and Ylitalo (1979) intravenously administered doses ranging from 10 to 250 mg/kg ^{14}C MCPA and ^{14}C
968 2,4-D to young and adult male Sprague-Dawley rats and determined the plasma and tissue distribution
969 of these constituents at various times following administration. Highest concentrations were achieved
970 approximately four hours following administration and declined thereafter with nearly complete
971 elimination at 120 hours. At four hours, the ^{14}C MCPA was nearly equally distributed between plasma,
972 kidney, and liver. A study of the intracellular distribution of 2,4-D across six organs in rats by Khanna
973 and Fang (1966) revealed that the soluble fraction of the cells contained the major portion of
974 radioactivity, followed by the nuclear fraction, and finally the mitochondrial and microsomal fractions.
975 Maximum plasma concentrations occurred within two to four hours of a 5 mg/kg orally administered
976 dose in rats (van Ravenzwaay et al. 2004). Bergesse and Balegno (1995) found that radio-labeled 2,4-D
977 uptake in Chinese hamster ovary cells was rapid and not metabolized. Uptake was pH-dependent and
978 reached a maximum at a pH of 4.5, falling to 5% of the maximum at a pH of 8.5, suggesting that uptake
979 would be limited at *in vivo* pHs.

980 4.1.3 Metabolism

981 2,4-D is excreted largely as parent compound and shows very little metabolism *in vivo* (van Ravenzwaay
982 et al. 2003). van Ravenzwaay et al. (2004) found oxidation of MCPA in rats exposed *in vivo*, and observed

983 largely unchanged levels of MCPA together with low levels of the oxidation product HMCPA (4-chloro-2-
984 hydroxymethylphenoxyacetic acid) in urine. Oxidation typically increases water solubility and therefore
985 excretion. Bacher and Gibson (1988) and Mustonen (1989) demonstrated the ability of MCPA and 2,4-D
986 to induce microsomal P-450 in rat liver, while Bergesse and Balegno (1995) demonstrated no metabolic
987 activity in Chinese hamster ovary cells exposed *in vitro* to pure 2,4-D. Observed responses were at
988 concentrations exceeding renal transport mechanisms.

989 4.1.4 Elimination

990 Elimination of both 2,4-D and MCPA following oral administration is rapid and complete, occurring
991 within 48 hours of exposure (van Ravenzwaay et al. 2003; 2004). Bellet et al. (1999) report that in
992 studies in rats, goats, and poultry, greater than 94% of the administered ¹⁴C MCPA acid was absorbed
993 and excreted unchanged in the urine within 24 to 48 h. Renal excretion is the key elimination
994 mechanism, and this relies on active tubular secretion and reabsorption with negligible glomerular
995 filtration (Bräunlich et al. 1989; Knopp 1994). Continued exposure (for example, occupationally) results
996 in steady-state exposures in which the amount excreted daily in urine is approximately equivalent to the
997 amount absorbed each day (Aylward and Hayes 2008; Knopp and Glass 1991; Knopp 1994).

998 Gorzinski et al. (1987) conducted a series of acute, pharmacokinetic, and subchronic toxicological
999 studies in rats involving technical grade 2,4-D acid, two forms of the salt, and four forms of the ester at
1000 doses ranging from 0 to 150 mg/kg-d. The concentration of ¹⁴C in plasma and the amount excreted in
1001 urine were proportional to dose up to doses of 50 mg/kg 2,4-D, but at 100 and 150 mg/kg, the
1002 concentration of ¹⁴C in plasma was greater than expected based on the lower doses indicating
1003 saturation of renal clearance mechanisms above approximately 50 mg/kg in the rat, similar to the
1004 results obtained by van Ravenzwaay et al. (2003).

1005 Lappin et al. (2002) orally administered ¹⁴C MCPA to rats and dogs at 5 or 100 mg/kg in order to explore
1006 differences in plasma toxicokinetics, rates and routes of excretion and biotransformation. Elimination of
1007 radioactivity was biphasic in rat plasma and monophasic in the dog. For both species, the principal route
1008 of excretion was via urine but renal elimination was notably more rapid and more extensive in the rat. In
1009 both rat and dog, excretion of radioactivity was mainly as MCPA and its hydroxylated metabolite
1010 (HMCPA). In the rat, both were mainly excreted as the free acids although a small proportion was
1011 conjugated. In the dog, the proportion of HMCPA was increased and the majority of both species was
1012 excreted as glycine or taurine conjugates. These data, along with previously published accounts, indicate
1013 that renal elimination of MCPA in dogs is substantially slower than in rats. These pharmacokinetic
1014 differences indicate that studies in dogs are not relevant for potential human health effects (Timchalk
1015 2004).

1016 Sauerhoff et al. (1977) conducted a study in five male human volunteers who ingested a single dose of 5
1017 mg/kg 2,4-D without detectable clinical effects. Concentration of 2,4-D was determined in plasma in
1018 three of five subjects and in urine in all subjects at timed intervals. The elimination of 2,4-D from plasma
1019 in all subjects occurred by an apparent first-order rate process with an average half-life of 11.6 h. All
1020 subjects excreted 2,4-D in the urine with an average half-life of 17.7 h. Excretion occurred mainly as 2,4-
1021 D (82.3%) with smaller amounts excreted as a 2,4-D conjugate (12.8%). Essentially all of the 2,4-D was
1022 absorbed from the gastrointestinal tract in man. No evidence of nonlinear kinetics was observed
1023 following the 5 mg/kg oral dose of 2,4-D.

1024 Knopp (1994) followed 27 men and 18 women over a five-year period (1985 – 1989) and measured
1025 urinary and serum levels in order to estimate excretion rates. Following five days of exposure during the
1026 work week, the author found that urinary concentrations decreased dramatically over a weekend of no

1027 exposure and returned to steady state during the following week of exposure, consistent with rapid
1028 clearance of 2,4-D from the body.

1029 **4.2 Pharmacodynamics**

1030
1031 Dierickx (1983) explored the *in vitro* interaction of 2,4-D, MCPA, MCPP, and 2,4-DP with rat-liver
1032 glutathione S-transferase (GST) using reduced glutathione and l-chloro-2,4-dinitrobenzene as substrates
1033 and found significant, dose-dependent inhibition of GST activity across compounds, albeit at
1034 concentrations of approximately 0.1 mM or 22 µg/ml, a plasma concentration saturating renal
1035 clearance. Ring substitution and side-chain length were shown to be of importance in determining the
1036 extent of GST inhibition. GST AA, an isoenzyme of GST, was stimulated by MCPA and 2,4-D. The author
1037 concludes that MCPA and 2,4-D interact with GST by binding directly to these proteins and this may
1038 have a protective function against these herbicides.

1039 Bukowska et al. (2003) explored the effects of exposure of human erythrocytes to different
1040 concentrations of MCPA and its environmental metabolite—2,4-dimethylphenol (2,4-DMP) with respect
1041 to glutathione content (GSH and GSSG), glutathione peroxidase (GSH-Px), glutathione transferase (GST),
1042 and the level of adenine energy charge (AEC). GSH protects cells from oxidative damage caused by free
1043 radicals. MCPA (250 ppm) decreased the level of GSH in erythrocytes by 9.2% and 2,4-DMP by 33.3% in
1044 comparison with controls at 250 and 500 ppm but not at lower concentrations, and this decrease was
1045 not statistically significant. Glutathione transferase activity was not altered for any compound across all
1046 concentrations tested.

1047 Palmeira et al. (1994a; 1994b; 1995a; 1995b) conducted a series of studies using rat hepatocytes and
1048 found that at concentrations starting at approximately 200 µg/ml, 2,4-D induced time and dose-
1049 dependent cell death accompanied by depletion of GSH.

1050 A significant fraction of the absorbed dose of 2,4-D and MCPA circulates in plasma before being
1051 excreted, or in the case of low-level chronic exposures, concentrations in plasma will reach steady state
1052 levels relative to exposures. Consequently, it is important to understand the potential effects of
1053 circulating 2,4-D and/or MCPA on cell structure and function. Several studies have evaluated the ability
1054 of 2,4-D, MCPA and other chlorophenoxy compounds to cause cellular damage that may be relevant to a
1055 toxic mode of action, including hemolysis, hemoglobin oxidation, and lipid peroxidation (Kozuka et al
1056 1991; Duchnowicz et al. 2002; 2005; Duchnowicz and Koter 2003; Saghir et al. 2006). The
1057 concentrations at which these kinds of effects are typically noted, however, tend to be above
1058 concentrations at which renal saturation occurs. Kozuka et al. (1991) examined the *in vivo* effects of
1059 MCPA, 2,4-D, and several other chlorophenoxy compounds on peroxisomal fatty acid oxidation-related
1060 enzymes in rat liver and found a significant increase in hepatic peroxisomal fatty acid oxidation in male
1061 Wistar rats orally exposed to 150 mg/kg-d 2,4-D for two weeks, while no effects were observed for
1062 MCPA, again, at concentrations exceeding renal saturation.

1063 In another series of studies to evaluate potential cellular damage caused by 2,4-D and MCPA and their
1064 metabolites, Duchnowicz et al. (2002; 2003; 2005) exposed human erythrocytes to concentrations of
1065 2,4-D and MCPA ranging from 1mM (200 – 221 µg/ml) to 4 mM (1,000 – 1,105 µg/ml). Effects, ranging
1066 from ATPase activity to lipid peroxidation, were only observed in a few instances at concentrations
1067 greater than 1mM and typically at concentrations greater than 4 mM. Hemolysis was not increased.
1068 Duchnowicz et al. (2005) found that exposure of human erythrocytes to 220 ppm 2,4-D and MCPA
1069 caused an increase in ATPase activity relative to controls that decreased relative to controls at higher
1070 tested concentrations (440 and 884 ppm); however, these high concentrations are not informative with
1071 respect to *in vivo* population exposures.

1072 Bukowska and Hutnik (2006) explored the effect of 2,4-D, MCPA, and the derivatives phenol, 2,4-
1073 dichlorophenol (2,4-DCP), 2,4-dimethylphenol (2,4-DMP), and catechol on the activity of
1074 acetylcholinesterase (AChE, EC3.1.1.7) in human erythrocytes. AChE activity is considered an indicator of
1075 the ability of an exposure to cause membrane damage. Phenol, MCPA, and 2,4-DMP did not significantly
1076 change AChE activity in human erythrocytes while decreases in AChE activity were observed under the
1077 highest applied dose of 2,4-D at 500 and 1000 ppm.

1078 Bukowska et al. (2008) investigated the effect of the sodium salt of 2,4-D (2,4-D-Na) and sodium salt of
1079 MCPA (MCPA-Na) on the oxidation of dihydrorhodamine 123 and H2DCFDA, carbonyl group content in
1080 cellular proteins, and hemoglobin denaturation. The rate of fluorescent probe oxidation was significantly
1081 higher for 2,4-D-Na, while both compounds increased the contents of protein carbonyl groups. No
1082 changes in the denaturation of hemoglobin were observed. 2,4-D-Na induced H2DCF oxidation in
1083 human erythrocytes in a linear dose-response up to four hours. MCPA-Na did not induce H2DCF
1084 oxidation even at the highest concentration during 3 h of incubation. Statistically significant changes
1085 were observed only for MCPA and 2,4-D at approximately 500 ppm following 24 h of incubation. The
1086 authors found that only 1% of the MCPA and 2,4-D used in this experiment penetrated the cell
1087 membrane, requiring significantly higher concentrations than would be experienced *in vivo* even
1088 occupationally and clearly exceeding renal transport mechanisms in humans.

1089 Bukowska et al. (2000) investigated the effect of 2,4-D on catalases in human erythrocytes at 100, 500
1090 and 1000 ppm over one hour, three hours and 24 hours. Catalases are important in eliminating the
1091 potentially dangerous formation of free radicals in cells, thus, a decline in catalase activity could be
1092 significant with respect to protecting cells. The authors found a small but statistically significant decline
1093 in catalase activity from exposure to 2,4-D and MCPA at the highest concentration (1000 ppm) but not
1094 at lower concentrations, and only after 3 hours for 2,4-D and 24 hours for MCPA. Similarly, Kaioumova

1095 et al. (2000) found that 2,4-D salt was able to cause apoptosis in peripheral blood lymphocytes of
1096 healthy individuals and Jurkat T cells in a dose and time dependent manner, but only at concentrations
1097 leading to acute poisoning effects *in vivo*.

1098 2,4-D and MCPA have both been shown to induce peroxisome proliferation (Vainio et al. al 1982;
1099 Timchalk 2004; Wetmore et al. 2011) but neither showed any agonistic activity via PPAR α in *in vitro*
1100 reporter gene assays with CV-1 cells (Takeuchi et al. 2006). Maloney and Waxman (1999) also reported
1101 that 2,4-D and MCPA were inactive in the human and mouse PPAR α activation assays using simian renal
1102 carcinoma COS-1 cells. Thus, although 2,4-D and MCPA have been shown to be peroxisome
1103 proliferators, they do not interact with PPAR α in cell culture systems, and *in vivo* conversion to more
1104 toxic metabolites, a potential pathway for induction of carcinogenic effects of peroxisome proliferation,
1105 has not been shown to occur. In addition, concentrations at which proliferation has been observed have
1106 been in excess of 100 ppm, well in excess of renal transport mechanisms in humans.

1107 Under the US EPA high-throughput screening ToxCast program, both MCPA and 2,4-D were screened in
1108 concentration-response format across more than 500 cell-based and biochemical assays (Wetmore et al.
1109 2011; Judson et al. 2010). Table 5 provides a brief description of the six positive assay results for MCPA
1110 and the eight positive assay results for 2,4-D. The level shown is the lowest effective concentration in
1111 μ M to elicit the response. A change in cell growth kinetics is the most sensitive assay for 2,4-D, while
1112 upregulation of CD38 expression is the most sensitive assay result for MCPA. CD38 is a transmembrane
1113 glycoprotein highly expressed in B-cell lymphoma ([http://www.ihop](http://www.ihop.net.org/UniPub/iHOP/gismo/87033.html)
1114 [net.org/UniPub/iHOP/gismo/87033.html](http://www.ihop.net.org/UniPub/iHOP/gismo/87033.html)) and is known to prevent apoptosis in germinal center B-cells.
1115 This may be a relevant pathway with respect to proliferation of key chromosomal aberrations.
1116 Significantly increased expression of CD38 has been observed across several NHL subtypes, although the
1117 mechanistic relevance is unknown.

1118 ToxCast assay results show that MCPA led to increased expression of CYP2B6, one of the P450 enzymes.

1119 De Roos et al. (2006) found increased risk for developing NHL for certain CYP variants, although they did
1120 not evaluate this specific variant.

1121 The change in cell growth kinetics observed for 2,4-D may also suggest a relevant pathway, although the
1122 specific change observed in the assay is not readily identifiable with respect to an *in vivo* response.

1123 Given that MCPA is a close structural analog to 2,4-D, one might have expected greater concordance
1124 across ToxCast assay results between the two compounds. The implications of differing ToxCast assay
1125 results remain unknown.

1126 **4.3 Summary of Pharmacokinetic and Pharmacodynamic Studies**

1127

1128 • 2,4-D and MCPA are readily absorbed and excreted largely as parent compound via active
1129 tubular transport and reabsorption

1130 • Saturation of renal clearance occurs at approximately 50 mg/kg in male rats, resulting in
1131 nonlinear increases in plasma concentrations

1132 • Both 2,4-D and MCPA undergo saturable and reversible protein (primarily albumin) binding

1133 • Approximately 10% or less of dermally applied doses are absorbed in humans (up to 20% in
1134 mice)

1135 • 2,4-D and MCPA induce P450 in rodents at concentrations that are readily excreted

1136 • Human erythrocytes incubated in greater than 110 µg/ml of MCPA and 2,4-D showed
1137 membrane damage, lipid peroxidation, and a decrease in –SH groups; translating to *in vivo*
1138 concentrations exceeding saturation of renal clearance (e.g., readily excreted)

1139 • 2,4-D and MCPA lead to cytotoxicity in hepatocytes and interact with liver mitochondrial
1140 energetics starting at approximately 200 µg/ml (e.g., concentrations exceeding saturation of
1141 renal clearance and therefore readily excreted)

- 1142 • 2,4-D and MCPA have been shown to be peroxisome proliferators at concentrations exceeding
1143 renal transport mechanisms, but do not generate metabolites and showed no agnostic activity
1144 via PPAR α
- 1145 • MCPA tested positive in only six of over 500 ToxCast assays and 2,4-D in eight; the assays related
1146 to cell growth and proliferation may be relevant for increased risk of lymphoma. However,
1147 there is little concordance across ToxCast results between the two compounds, and
1148 corresponding *in vivo* concentrations are high

1149 **5.0 Toxicological Studies**

1150 This section summarizes the available literature for toxicological studies, either conducted *in vivo* or *in*
1151 *vitro* in animals, or using *in vitro* human cell cultures or through *in vivo* human exposures. Table 6
1152 provides a summary of the *in vivo* animal studies for 2,4-D and MCPA, while Table 7 focuses on *in vitro*
1153 studies using non-human cell cultures. Table 8 provides a summary of studies based on either *in vivo*
1154 human exposures or *in vitro* human cell cultures. Studies are discussed in the context of the potential
1155 modes of action.

1156 Toxicological studies provide an important link back to the observational studies in humans by allowing
1157 for a more detailed evaluation of hypothesized modes of action. What is difficult here with respect to
1158 exposures to 2,4-D and/or MCPA is that none of the standard rodent bioassays demonstrate
1159 carcinogenicity of these compounds. In evaluating the potential for exposures to lead to carcinogenic
1160 outcomes, generally there is a discussion of the concordance across outcomes in animals and humans,
1161 and the standard *in vivo* toxicological rodent bioassays represent an important link between potential
1162 effects in animals versus humans. But in this case, there are no relevant tumorigenic outcomes in
1163 animals based on a series of assays conducted in support of the pesticide registration process. There are
1164 a number of assays and observations *in vivo* and *in vitro* at the cellular level (e.g., sister chromatid

1165 exchange, micronucleus formation, etc) that in some cases do show negative responses following
1166 exposures, but in the absence of frank tumor formation in rodents using standard multi-year and multi-
1167 generational assays, the implication of those results require cautious interpretation.

1168 Initial candidate studies are identified through an online search of PubMed, Medline, and Web of
1169 Science using search terms including “chlorophenox*” and “health” and “effect” or “toxicol*”. Further
1170 studies are identified through careful review of the reference lists of studies obtained through the
1171 literature search, and of the reference lists of prior reviews (particularly for the older studies).

1172 Toxicological studies are evaluated using the following set of questions:

1173 *Does the assay involve in vivo human exposures or human cell lines?* Given the lack of tumorigenic
1174 responses across standard rodent bioassays, this evaluation considers *in vivo* and *in vitro* responses in
1175 humans and human cell systems to be more relevant than responses in animal systems. For example, a
1176 study documenting cellular responses following *in vivo* exposures is given greater weight than a strictly
1177 *in vitro* study or a similar study in animals.

1178 *Is there evidence for direct DNA interaction, and if so, based on which assays?* Butterworth (2006)
1179 outlines a battery of typical *in vivo* and *in vitro* tests and provides a rationale for evaluating the results of
1180 specific assays. For example, results from bacterial mutagenicity, *in vivo* mouse bone marrow
1181 micronucleus tests, and human lymphocyte chromosomal aberration assay results should be considered
1182 to provide stronger evidence of mutagenic potential than the CHO chromosomal aberration assay and
1183 the mouse lymphoma mutagenicity assay because of the high false positive rate of the latter two assays.
1184 Moreover, the combination of positive assays and cell proliferation provides stronger evidence of
1185 potential carcinogenicity as DNA replication is required to convert a DNA adduct into a permanent
1186 mutation. A genotoxic chemical administered at a toxic dose that also induces cell proliferation will be

1187 far more effective as a mutagen and as a carcinogen than when given at a low dose that does not induce
1188 cell proliferation (Butterworth 2006; Matthews et al. 2006).

1189 *Is there evidence for non-DNA reactive carcinogenicity?* Chronic inflammation, nuclease release,
1190 sustained stimulation of regenerative cell proliferation, and interruptions to cell cycle regulation and
1191 function all provide evidence of a non-DNA reactive mode of action (Matthews et al. 2006).

1192 *What are the in vivo exposures implied by the study and how do those relate to known exposures from*
1193 *the biomonitoring and PBPK studies?* Particularly with respect to the *in vitro* studies, the concentrations
1194 at which responses are observed can translate to *in vivo* exposures that exceed systemic toxicity and/or
1195 renal clearance mechanisms, or that require exposures that are much greater than occur during typical
1196 product use. A key element here is the fact that dermal exposures are the predominant exposure
1197 pathway given chlorophenoxy use and properties, and studies show that approximately 10% of a
1198 dermally applied dose is absorbed (Ross et al. 2005). It is then possible to ask how much of the
1199 constituent would need to be sprayed or used to lead to a dermal exposure at which effects might be
1200 observed, and do the data suggest these kinds of exposures are realistic given what is known about
1201 product use.

1202 **5.1 Toxicological Studies in Animals**

1203 In general, standard two-year or multi-generational *in vivo* toxicological studies in rats and mice show
1204 virtually no evidence of treatment-associated carcinogenicity, and very little in the way of histological
1205 effects resulting from sustained exposure to these constituents. The primary effects noted *in vivo* relate
1206 to organ weights and some limited hyperplasia. The US EPA human health risk assessment developed to
1207 support the reregistration decision of MCPA finds that MCPA is “not mutagenic” and “unlikely to be a
1208 carcinogen” (US EPA 2004a), while it considers 2,4-D “unclassifiable” with respect to carcinogenicity (US
1209 EPA 2005). Accordingly, there are no published slope factors in IRIS for either constituent.

1210 Section 5.1.1 provides a brief summary of the standard rodent bioassays, while Section 5.1.2 presents
1211 the data for genotoxicity in animal systems. Cytotoxicity and related cellular effects are discussed in
1212 Section 5.1.3.

1213 5.1.1 Rodent Bioassays

1214 5.1.1.1 MCPA

1215 The published RfD for MCPA is 0.0005 mg/kg-day based on a study of technical grade MCPA orally
1216 administered to male and female beagle dogs (6/sex/dose) at doses of 0, 6, 30, or 150 ppm (0, 0.15,
1217 0.75, or 3.75 mg/kg/day) for 52 weeks (unpublished 2,4-D Task Force study). This dosage resulted in
1218 kidney and liver toxicity at the mid- and/or high-dose levels, with alterations in clinical chemistries
1219 (kidneys: urea, potassium, creatinine; liver: bilirubin, GPT, GOT, triglycerides, and cholesterol) associated
1220 with concomitant organ weight changes (liver) and histopathology changes (kidney: increased kidney
1221 pigment deposition in proximal tubular epithelium; liver: change in the nature/coloration of gall fluid).
1222 Therefore, based upon kidney and liver toxicity at the 30 and 150 ppm dose levels, the lowest effect
1223 level for systemic toxicity was determined to be 0.75 mg/kg/day. The NOEL for systemic toxicity was
1224 estimated at 0.15 mg/kg/day, with an uncertainty factor of 300 to obtain the RfD.

1225 Bellet et al. (1999) report on two chronic toxicity/oncogenicity studies. In the first, MCPA was
1226 administered to 50 male and 50 female Wistar rats at doses of 0, 20, 80, and 320 ppm in the diet for 24
1227 months. There was no effect observed on mortality in any dose group. Responses noted at 320 ppm
1228 included slight but statistically significant decreases in body weights in males as compared to controls.
1229 Small changes in clinical chemistry parameters were also noted in males as compared to females from
1230 the same dose group. Spontaneously occurring nephropathy was more pronounced in male rats in the
1231 320 ppm dose group, including an increase in the retraction and granular surface of the kidneys. In
1232 female rats, a statistically significantly higher mean absolute kidney weight of female rats from the 80-

1233 ppm dose group was noted. However, there was no weight change in the high-dose females, and no
1234 histological effects observed in female rats at any dose level. The systemic NOEL was estimated at 20
1235 ppm (approximately 1.3 mg/kg/day) for both male and female rats based upon the nephrotoxicity
1236 observed in either the 80 or the 320 ppm dose groups. No carcinogenic or oncogenic responses were
1237 observed across all doses.

1238 In the second study, MCPA was administered in the diets of 50 male and 50 female B6C3F1 mice at
1239 doses of 0, 20, 100, or 500 ppm over a two-year period. There was no effect observed on mortality
1240 across dose groups. Reduced body weight gain was noted throughout the study in male mice receiving
1241 500 ppm MCPA, except for the last six months. Both absolute and relative kidney weights showed a
1242 statistically significant increase in female mice from the high-dose group, which was associated with
1243 histopathological changes noted in the kidneys in both sexes. Male and female mice in the highest dose
1244 group showed an increased incidence of intratubular calcification and tubular hyaline-proteinaceous
1245 casts. Male mice only showed a statistically significant increase in the incidence of renal tubular
1246 epithelial focal hyperplasia in the 500 ppm dose group. The systemic NOEL was estimated at 100 ppm.
1247 No carcinogenic or oncogenic responses were observed across all doses.

1248 *5.1.1.2 2,4-D*

1249 The published Reference Dose (RfD) for 2,4-D in the US EPA Integrated Risk Information System (IRIS;
1250 www.epa.gov/iris) is 0.01 mg/kg-d based on a no observed adverse effect level of 1 mg/kg-d from a 13-
1251 week subchronic study in rats. At 5 mg/kg-d, there were statistically significant reductions in mean
1252 hemoglobin (both sexes), mean hematocrit and red blood cell levels (both sexes), and mean reticulocyte
1253 levels (males only) after seven weeks. There were also statistically significant reductions in liver enzymes
1254 LDH, SGOT, SGPT, and alkaline phosphatase at week 14 in animals treated at the 15.0 mg/kg-day or
1255 higher doses. This RfD is currently in review.

1256 The U.S. EPA Office of Pesticide Programs used an RfD of 0.005 mg/kg-d based on an observed NOAEL of
1257 5 mg/kg-d as documented in the reregistration document (US EPA 2005, p. 21).

1258 Charles et al. (1996a; 1996b; 1996c) conducted a series of chronic and subchronic toxicity studies using
1259 all three forms of 2,4-D (the acid parent compound, the salt and the ester). The first chronic toxicity
1260 study in male and female Fischer rats found no effects of exposure. The subchronic study (13 weeks)
1261 found decreased red blood cell and platelet counts, and decreased circulating T3 and T4 levels at the
1262 100 mg/kg-d dose level. In contrast, Gorzinski et al. (1987) in a 13-week subchronic study with rats
1263 found statistically significantly decreased T4 levels at 100 mg/kg-d, but statistically significantly
1264 increased T4 levels at 15 mg/kg-d.

1265 Charles et al. (1996c) reported no changes across immunotoxicological parameters including bone
1266 marrow and/or lymph node histopathology or leukocyte counts in beagle dogs in a one-year feeding
1267 study with doses ranging from 1 mg/kg-d to 7.5 mg/kg-d. The subchronic (13 weeks) portion of the study
1268 established a NOEL of 1 mg/kg-d. Increases in blood urea nitrogen, creatinine, and ALT were
1269 consistently observed across the studies. With respect to chronic effects, the study noted no other
1270 effects at one year that weren't already evident at 13 weeks; thus, the authors argue for a chronic NOEL
1271 of 1 mg/kg-d based on this study.

1272 Blakley et al. (1992) exposed male CD-1 to a commercial amine derivative of 2,4-D in drinking water to
1273 evaluate the effect of 2,4-D on the incidence of spontaneous murine lymphocytic leukemia over a 365
1274 day treatment period and found that mortality associated with the leukemia was not impacted in any
1275 way by the 2,4-D treatment. In another study, Blakley et al. (1998) evaluated immune function in male
1276 Fischer 344 rats exposed to 10 mg/kg of 2,4-D by oral gavage twice weekly for four weeks and found no
1277 effect on lymphocyte glastogenesis, lymphocyte cell surface marker expression or phagocytic function of
1278 peritoneal macrophages.

1279 The only evidence for carcinogenicity of 2,4-D in animal studies is based on a case-control study of
1280 pathologically confirmed cases of lymphoma in dogs (Hayes et al. 1991). This study was subsequently
1281 reviewed by an expert panel (Carlo et al. 1992), who identified significant limitations associated with the
1282 study and concluded no association between 2,4-D exposure and canine lymphoma given similar
1283 limitations as the human epidemiologic studies (e.g., exposures were poorly understood and specific
1284 exposures only qualitatively identified, no observed dose-response relationship, etc.). Kaneene and
1285 Miller (1999) re-analyzed the Hayes et al. (1991) data using the exposure definition used in the original
1286 study, re-analyzed the data using a redefinition of exposure, and conducted a dose-response analysis
1287 with the redefined exposure criteria, and did not confirm a dose-response relationship between 2,4-D
1288 use and lymphoma in dogs. The re-analysis found no significant association between 2,4-D exposure and
1289 canine lymphoma.

1290 Reynolds et al. (1994) showed that dogs exposed to lawns following various types of herbicide
1291 treatment do show measurable urinary levels of 2,4-D (on the order of 10 µg/L and some as high as 50
1292 µg/L ten days following application). A recent study (Takashima-Uebelhoer et al. 2012) found a
1293 statistically-significant association between the use of "self-applied insect growth regulators" (OR = 2.7, 95%
1294 CI = 1.1 - 6.8) and canine lymphoma, but specific constituents were not identified. Associations between
1295 herbicides were not statistically significant (OR = 1.3, 95% CI = 0.9 - 1.8).

1296 The only available study that evaluated any effects following dermal exposures is Schop et al. (1990)
1297 who exposed male CD1 mice dermally to 500, 1000, and 2000 µMol/kg-day (approximately 110, 220,
1298 and 442 ppm, respectively). They compared the results of the bone marrow micronucleus test and hair
1299 follicle nuclear aberration assay conducted 24 hours following topical application of 2,4-D and found no
1300 effect in the micronucleus test, and a statistically significant increase of a 2% increase relative to
1301 controls in the nuclear aberration assay (at the site of exposure). Concentrations of 2,4-D were not
1302 measured in the animals; however, Ross et al. (2005) report that mice show amongst the highest dermal

1303 absorption proportion relative to exposed dose (20%). Consequently, the 2,4-D should have been
1304 completely absorbed in 24 hours.

1305 5.1.2 Genotoxicity Based on *in vitro* or *in vivo* Animal Studies

1306 A hypothesized mode of action is that exposure to 2,4-D and/or MCPA leads to direct DNA damage.
1307 Specific chromosomal translocations have been observed across the lymphomas, particularly NHL, and
1308 evidence is increasing that these are a prerequisite for disease. This section evaluates the data on
1309 genotoxicity for these two compounds based on laboratory studies in animals.

1310 5.1.2.1 MCPA

1311 Bond and Rossbacher (1993) report that MCPA did not cause point mutations when tested in the Ames
1312 test or the host mediated assay or in mammalian (V79) cells. No increase occurred in chromosomal
1313 aberrations in Chinese hamster bone marrow cells after oral exposure of the animals. A weak increase in
1314 the rate of SCE was found in the same animal strain at toxic doses *in vivo*, whereas at lower doses there
1315 were no adverse effects observed. A DNA-binding study of radiolabelled MCPA did not show any
1316 interaction of the compound with the genetic material of the liver cells. Other test systems showed
1317 equivocal results (SLRL test, assays in yeast cells). Considering all mutagenic studies carried out with
1318 MCPA, it can be concluded that most tests were negative, but that in some tests a weak mutagenic
1319 potential was found at doses that would lead to acute toxicity *in vivo*.

1320 Elliott (2005) similarly conducted a review of available *in vivo* (n=12) and *in vitro* (n=13) assays to
1321 evaluate the mutagenic and genotoxic potential of the amine salt-form of MCPA. Elliott concludes that
1322 MCPA is non-mutagenic across bacterial and mammalian cell gene mutation assays. He notes increases
1323 in percentage aberrant cells found on analysis of metaphases of human peripheral lymphocytes treated
1324 *in vitro* in the presence of auxiliary metabolic activation (S9), but only at doses approaching 10 mM and

1325 leading to significant cytotoxicity. The fact that metabolic activation was required suggests that this
1326 effect would not be noted *in vivo*. No evidence for clastogenicity *in vivo* was found in the mouse bone
1327 marrow micronucleus assay or the Chinese hamster bone marrow metaphase assay. No evidence for
1328 either increases in SCE frequency or DNA binding was found in the rat. Very small (less than 1.5 times
1329 controls) increases in SCE were observed *in vivo* in the hamster at toxic or maximum tolerated dose
1330 levels. Elliott (2005) concludes there is no *in vivo* or *in vitro* evidence for mutagenicity of MCPA,
1331 particularly the salt forms of the compound typically used in commercial products.

1332 5.1.2.2 2,4-D

1333 Amer and Aly (2001) treated six Swiss mice by oral gavage with 2,4-D at 1.7, 3.3 and 33 mg/kg. 2,4-DCP,
1334 the 2,4-D metabolite, was intraperitoneally injected at 36, 72 and 180 mg/kg. Oral treatment by gavage
1335 with the lowest tested dose (1.7 mg 2,4-D kg⁻¹ BW) for five consecutive days had no significant effect
1336 on the induction of chromosomal aberrations, but a significant increase in the percentage of
1337 chromosomal aberrations in bone-marrow and spermatocyte cells was observed after oral
1338 administration of 2,4-D at 3.3 mg/kg bw for three and five consecutive days. The number of observed
1339 chromosomal aberrations was a factor of four higher for the positive control injected with mytomicin C
1340 as compared to the 2,4-D exposed animals, and the number of chromosomal aberrations was not dose-
1341 dependent. 2,4-DCP injected animals only showed a response at the highest dose tested (180 mg/kg).

1342 Charles et al. (1999a) conducted *in vitro* unscheduled DNA synthesis assays on male Fischer 344 rat
1343 hepatocytes using parent 2,4-D compound and seven derivatives of salts, esters, and amines. Plate
1344 concentrations ranged from 2 – 340 µg/L and no effects were observed. In the same study, the authors
1345 also conducted the Ames bacterial reverse mutation assay (including a positive control) and found no
1346 effects across all treatment types. A follow-on study (Charles et al. 1999b) evaluated the potential for
1347 2,4-D and seven of its salts and esters to induce cytogenetic abnormalities in mammalian cells *in vivo*

1348 using the mouse bone marrow micronucleus test in CD-1 mice. All the test materials were administered
1349 to male and female mice by oral gavage and the frequencies of micronucleated polychromatic
1350 erythrocytes MN-PCE in bone marrow were determined at intervals of 24, 48 and 72 h following dosing.
1351 There were no significant increases in the incidence of MN-PCE in the treated mice at any of the bone
1352 marrow sampling times. Five animals per group were sacrificed at either 2–4 h, or 12–14 h, after dosing.
1353 Treatment with 2,4-D at doses up to 1000 mg/kg demonstrated no effects on rat hepatocytes.

1354 Maire et al. (2007) After 5 h of treatment, the percentage of SHE cells with damaged DNA was 8% in
1355 class 1, and 0.7% in class 2 (percentage of DNA in the tail between 40% and 60%) after exposure to
1356 11.5M 2,4-D. After 5 h of treatment at 23M, the percentage of DNA-damaged cells was 12.3% in class 1
1357 and 1.3% in class 2. After 24 h of treatment at 11.5M 2,4-D, 9.7% of cells ranked in class 1 and 0.3% in
1358 class 2. At 11.5 M 2,4-D, 17% of cells ranked in class 1, and 5.3% in class 2, while 1.3% of cells were in
1359 class 3 with a high level of DNA breaks (percentage of DNA in the tail between 60% and 80%). After 2 h
1360 of treatment with the positive control H₂O₂ (500M), the percentage of SHE cells with DNA damage was
1361 18% in class 1, 10% in class 2, while 67% of the cells were in class 3, with a high level of DNA breaks.

1362 Gollapudi et al. (1999) investigated the genetic toxicity of 2,4-D 2-butoxyethylester and two salts (2,4-D
1363 isopropylamine and 2,4-D triisopropanolamine) in cultured Sprague Dawley rat cells. The end points
1364 used were the induction of chromosomal aberrations in primary cultures of rat lymphocytes and
1365 forward mutations at the HGPRT locus of Chinese hamster ovary (CHO) cells with and without S9
1366 activation. There was no evidence of genotoxicity across test materials. However, Gonzalez et al. (2005)
1367 evaluated the potential genotoxicity of 2,4-D and a commercially-used derivative, 2,4-D dimethylamine
1368 salt (2,4-D DMA) in CHO cells using SCE and single cell gel electrophoresis (SCGE) assays and found
1369 significant dose-dependent increases in SCE, regardless of the harvesting time (2,4-D: $r = 0.98$ and $r =$
1370 0.88 , $P < 0.01$, for 24 and 36h harvesting times; 2,4-D DMA: $r = 0.97$ and $r = 0.88$, $P < 0.01$, for 24 and

1371 36h harvesting times). Log-phase cells were treated with 2.0–10.0 µg/ml of herbicides and harvested 24
1372 and 36h later for SCE analysis. Neither test compound altered cell-cycle progression or proliferative
1373 replication index ($P > 0.05$), but the higher doses of both compounds reduced the mitotic index of
1374 cultures harvested at 24 and 36h ($P < 0.05$). A 90-min treatment with 2.0–10.0 µg/ml 2,4-D and 2,4-D
1375 DMA produced dose-dependent increases in the frequency of DNA-strand breaks detected in the SCGE
1376 assay, both in cultures harvested immediately after treatment and in cultures harvested 36h later. The
1377 doses of 2,4-D and 2,4-D DMA were equally genotoxic in all of the assays. By contrast, Linnainmaa
1378 (1984) reported no increase in SCE frequency after a 1h pulse-treatment of CHO cells with pure 2,4-D
1379 and a commercial 2,4-D formulation (2,4-D amine salt as the active ingredient) with and without S9
1380 activation.

1381 Gonzalez et al. (2005) evaluated the potential genotoxicity of pure 2,4-D (acid) and the commercially
1382 used salt in Chinese hamster ovary cells treated with 2.0 – 10.0 µg/ml using SCE and single cell gel
1383 electrophoresis (SCGE) assays. The authors found that both forms of 2,4-D induced significant dose-
1384 dependent increases in SCE, but neither test compound altered cell-cycle progression or replicative
1385 index. The highest doses of both forms of 2,4-D reduced the mitotic index of cells.

1386 **5.2 Studies in Humans and Using Human Cell Cultures**

1387 Table 8 summarizes the available data for assays involving human cell cultures or cells from humans
1388 exposed *in vitro* and *in vivo*. Several studies, particularly those based on field exposures *in vivo*, do not
1389 explicitly distinguish between MCPA and 2,4-D exposures and results are considered applicable to both.
1390 In general, observing an *in vivo* effect in humans takes precedence, although lack of an effect *in vivo*
1391 does not negate a positive *in vitro* effect. At that point, it is important to consider the conditions under
1392 which exposure across test systems, and how those relate to environmental exposures.

1393 **5.2.1 Genotoxicity**

1394 Mustonen et al. (1986) evaluated chromosomal aberrations *in vivo* in lymphocyte cultures from 19
1395 exposed 2,4-D and MCPA Swedish forestry sprayers. Workers sprayed 333 g/l 2,4-D and/or 167 g/l
1396 MCPA during July through October 1981 for a minimum of six days and a maximum of 28 days. No
1397 increase in the incidence of chromosomal aberrations in the lymphocytes of workers was observed in
1398 this study. These authors also conducted an *in vitro* study in which human peripheral lymphocytes were
1399 cultured with 0.125, 0.250, 0.500, 1.000 and 1.250 mM of pure 2,4-D as well as a commercial herbicide
1400 containing 2,4-D (*Vesakontuho Tasku* containing 550 g/l 2,4-D as amine salt in water). The pure 2,4-D
1401 product showed no induction of chromosomal aberrations of any kind but the commercial mixture
1402 showed statistically significant differences from controls in a dose-dependent manner starting at 0.5
1403 mM (110 ppm). The authors suggest this is due to impurities and phenols contained in the commercial
1404 mixture. However, Clausen et al. (1990) and Jacobi and Witte (1991) in separate studies involving a
1405 commercial formulation of 2,4-D argue that observed differences in toxicity may be attributable to
1406 differences in chemical structure between the pure acid and the soluble salt, although this seems
1407 unlikely as the soluble salt disassociates to the pure acid under physiological conditions.

1408 In an *in vivo* study in forestry workers spraying foliage with either 2,4-D, MCPA, or a mixture, Linnainmaa
1409 (1983) found no induction of SCEs in peripheral lymphocytes. SCE analyses were conducted on cells from
1410 35 herbicide workers and 15 control subjects. No statistically significant differences in the frequencies of
1411 SCEs were observed in samples taken before, during, or after the exposure, and the mean SCE from
1412 nonexposed control group fell in the same range as those of the exposed subjects.

1413 5.2.1.1 MCPA

1414 Elliot (2005) conducted a literature review of available genotoxicity and mutagenicity studies involving
1415 MCPA and find no evidence of these effects in human cell cultures. MCPA was not genotoxic in a

1416 battery of assays developed under the US EPA high-throughput screening ToxCast program (Knight et al.
1417 2009).

1418 5.2.1.2 2,4-D

1419 Korte and Jalal (1982) evaluated the clastogenic and mutagenic potential of 2,4-D in cultured
1420 lymphocytes. Chromosomal damage, though statistically insignificant, occurred at doses as low as 0.2
1421 µg/ml and increased at a statistically significant level at concentrations of 50 µg/ml or higher. Potential
1422 mutagenicity, based on rates of increase in SCE, was significant at 10 µg/ml or higher concentrations. In
1423 a similar study, Turkula and Jalal (1985) observed a weak increase in SCE in peripheral human
1424 lymphocytes exposed *in vitro* at 50, 100, and 250 µg/ml but the difference was only statistically
1425 significant at the lowest dose and the increase was less at higher doses than at the lowest dose.

1426 Soloneski et al. (2007) explored the genotoxic potential of 2,4-D and its commercial derivative 2,4-D
1427 DMA by measuring sister chromatid exchange (SCE), cell cycle progression and mitotic index in human
1428 whole blood (WBC) and plasma leukocyte cultures (PLC). Cells were exposed to concentrations of 10, 25,
1429 50 and 100 µg/ml for 72 h. SCE frequency was statistically significant increased at concentrations of 10
1430 to 50 µg/ml for 2,4-D and at 25 to 100 µg/ml for 2,4-D DMA. However, in PLC, there was no observed
1431 increase in SCE. A significant delay in cell proliferation was observed in WBC after treatments with 25
1432 and 50 µg/ml 2,4-D and 50 and 100 µg/ml 2,4-D DMA, whereas in PLC, only 100 µg/ml 2,4-D altered
1433 cell-cycle progression. For both chemicals, a progressive dose-related inhibition of mitotic activity was
1434 observed. The results demonstrated that the presence of erythrocytes in the culture system appeared to
1435 increase DNA and cellular damage inflicted by 2,4-D and 2,4-D DMA. However, again these
1436 concentrations are high relative to environmental exposures.

1437 Under the USEPA ToxCast program, a suite of chemicals, including 2,4-D, was tested in 467 assays
1438 including assays for genotoxicity (Judson et al. 2010) and found not to be genotoxic across a suite of

1439 assays (Knight et al. 2009). The U.S. EPA has reviewed the potential genotoxicity and mutagenicity of
1440 2,4-D (U.S. EPA 1994; 1997), most recently in 2012 (77FR23125). Those data show no evidence for
1441 heritable mutagenic effects in mammals but some evidence supporting 2,4-D's potential to cause
1442 genotoxic effects. Specifically, U.S. EPA concluded that the combined evidence shows: (1) 2,4-D is
1443 negative across bacterial mutation assays; (2) some positive results for mutagenicity in assays in yeast,
1444 plants, and insects; (3) negative results for mutagenicity based on *in vivo* mammalian studies; and (4)
1445 mixed results for mutagenic and genotoxic results based on mammalian *in vitro* tests.

1446 5.2.2 Proliferative and Immunological Effects

1447 In a study involving ten farmers who mixed and applied 2,4-D and MCPA for one to three days, Faustini
1448 et al. (1996) collected blood samples from ten farmers within seven days prior to exposure to 2,4-D.
1449 Samples were collected again one to 12 days after exposure, and again 50 to 70 days after exposure.
1450 Whole blood was used to count lymphocyte subsets with monoclonal antibodies. Peripheral blood
1451 mononuclear (PBM) cells were used to measure natural killer (NK) cell activity and lymphocyte response
1452 to mitogenic stimulations. Individual values collected prior to exposure were used as reference. Relative
1453 to concentrations prior to exposure, a significant reduction was found one to 12 days after exposure in
1454 the following variables ($P < 0.05$): circulating helper (CD4) and suppressor T cells (CD8), CD8 dim,
1455 cytotoxic T lymphocytes (CTL), natural killer cells (NK), and CD8 cells expressing the surface antigens
1456 HLA-DR (CD8-DR), and lymphoproliferative response to mitogen stimulations. All immunological values
1457 found 50-70 days after exposure were comparable with concentrations before exposure, with the
1458 exception of the percentage of CD8-DR cells, which continued to be statistically significantly decreased.
1459 Although exposures to chlorophenoxy compounds are episodic, there may be long term implications
1460 associated with repeated, short-term immunosuppression in cancer etiology. No correlation was found

1461 between kg of pesticide applied (which ranged from 12 to 155 kg across the ten participants) and
1462 immunological measures.

1463 5.2.2.3 MCPA

1464 Elliot (2005) reports on three studies using MCPA and peripheral human lymphocytes *in vitro* in which
1465 demonstrated cell cycle delays at concentrations greater than 500 µg/ml (original studies were not
1466 available from the primary literature).

1467 5.2.2.4 2,4-D

1468 Tuschl and Schwab (2003) and Kaioumova et al. (2001) were able to induce apoptosis by exposing
1469 HepG2 cells and human lymphocytes, respectively, *in vitro* for several days. However, these effects
1470 were only observed at high concentrations (above 884 µg/ml and 660 µg/ml, respectively). In theory,
1471 induction of apoptosis could be beneficial in individuals with an existing t(14;18) translocation since that
1472 leads to inhibition of apoptosis. However, these concentrations are too high to be relevant to *in vivo*
1473 exposures (Aylward and Hayes 2008).

1474 Figgs et al. (2000) found that the lymphocyte replicative index increased after spraying 2,4-D ($p = 0.016$),
1475 independent of tobacco and alcohol use, in a study involving two applicators spraying only 2,4-D. The
1476 data demonstrated a weak dose-response with increasing urinary 2,4-D levels ($p = 0.15$). Lymphocyte
1477 immunologic phenotypes and complete blood counts (CBC) before spraying 2,4-D were not statistically
1478 different after spraying 2,4-D, nor were there significant differences between 2,4-D applicators and
1479 controls after applicators had sprayed. The authors found no relationship between the frequency of
1480 micronuclei and urinary 2,4-D levels, and conclude there are no human chromosome-damage outcomes
1481 at mean urinary 2,4-D levels ranging from 12 to 1285 ppb. Increased replicative index scores may be
1482 important because they suggest stimulated cell growth that could contribute to carcinogenesis.

1483 However, the finding of no relationship between the frequency of micronuclei and urinary 2,4-D level
1484 does not support a human chromosome-damage outcome at mean urinary 2,4-D levels ranging from 12
1485 to 1285 ppb.

1486 In a follow-on study to Figs et al. (2000), Holland et al. (2002) evaluated cultured lymphocytes from the
1487 workers described above using a micronucleus assay and replicative index, a measure of cell division
1488 kinetics, as well as an associated *in vitro* study using whole blood and cultured lymphocytes to which a
1489 commercial formulation containing 2,4-D (Spurge and Oxalis Killer) as well as pure 2,4-D in different
1490 vehicles (e.g., ethanol, DMSO) was added. This study demonstrated that the lymphocytes of the 12
1491 male applicators described above had a significantly higher replicative index than the same group prior
1492 to exposure and than a control group ($P < 0.01$). These results corroborate the *in vitro* finding in this
1493 study of increased replicative index at low doses (0.005 mM 2,4-D). *In vitro* there was a significant
1494 inhibition of lymphocyte proliferation for all five individuals at the highest dose level (0.3 mM)
1495 independent of the vehicle used for both pure and commercial 2,4-D ($P < 0.001$). At the low dose (0.005
1496 mM) of commercial 2,4-D, four out of five study subjects exhibited an increase in replicative index. Pure
1497 2,4-D results were inconclusive with three individuals responding with increased proliferation and four
1498 actually declining. This study showed a micronucleus increase above normal baseline only at high 2,4-D
1499 doses, i.e. those approaching cytotoxic levels. The authors conclude that genotoxicity of 2,4-D as
1500 measured by the bone marrow micronucleus assay at environmentally relevant concentrations is
1501 negligible, but find that increased proliferation after low 2,4-D exposure may be significant. Similarly, an
1502 extensive review of 2,4-D by the German Research Foundation (Henschler and Greim 1998, p. 90)
1503 concluded there was sufficient evidence of a weak promoting effect of herbicide formulations of 2,4-D.

1504 **5.3 Summary of Toxicological Studies**

1505
1506 Table 9 provides a brief summary of the studies that have explored genotoxicity and cytotoxicity both *in*
1507 *vivo* and *in vitro* in animals and humans. A negative sign indicates a negative result. A single plus
1508 indicates a positive result, but either only weakly positive (not statistically significant) or statistically
1509 significant but at very high concentrations relative to environmental exposures, including occupational
1510 exposures.

1511 Rodent Bioassays

- 1512 • Standard carcinogenic bioassays in rodents show no carcinogenic effects at concentrations
1513 ranging from 1 to 500 mg/kg-d
 - 1514 ○ USEPA (IRIS) RfD for 2,4-D is 0.01 mg/kg-d (www.epa.gov/iris); the USEPA Office of
1515 Pesticide Programs uses 0.005 mg/kg-d in risk assessments conducted to support
1516 pesticide registration evaluations
 - 1517 ○ The published IRIS value is 0.0005 mg/kg-d; the USEPA Office of Pesticide Programs uses
1518 0.0044 mg/kg-d in risk assessments conducted to support pesticide registration
1519 evaluations

1520 Genotoxicity

- 1521 • Soloneski et al. (2007) and Zeljezic and Garaj-Vrhovac (2004) show that *in vitro* exposures at 4 –
1522 10 µg/ml 2,4-D and/or a commercial product containing 2,4-D are associated with statistically
1523 significant increases in SCE, but *in vivo* exposures in workers are more equivocal
- 1524 • Most *in vitro* studies in both human and animal cell cultures show effects at concentrations
1525 greater than would be expected in the environment
- 1526 • Genotoxicity was not observed across a battery of ToxCast assays

- 1527 • 2,4-D is negative for genotoxicity in bacterial mutation assays
- 1528 • Some positive results for mutagenicity have been observed in assays in yeast, plants, and insects
- 1529 • Negative results have been observed for mutagenicity across *in vivo* mammalian studies
- 1530 • Mixed results have been observed based on mammalian *in vitro* tests.

1531 Proliferative and Immunological Effects

- 1532 • 2,4-D and MCPA are weak peroxisome proliferators
- 1533 • 2,4-D and MCPA increase lymphocyte replicative index
- 1534 • Occupationally-exposed individuals showed temporary increases in immunological markers
- 1535 • MCPA tested positive in six of over 500 ToxCast assays, and 2,4-D tested positive in eight

1536 **6.0 Exposure and Biomonitoring**

1537 Although the dose makes the poison, it is the exposure that makes the dose, which is both a function of
1538 exposure concentrations in the environment and the relationship between exposed and absorbed dose.
1539 2,4-D and MCPA are relatively straightforward to study since they do not metabolize and are readily
1540 excreted in urine largely as parent compound within days of exposure. A number of models have been
1541 developed to explore and predict the relationship between exposures, particularly as defined in
1542 epidemiological studies (e.g., different spraying methods, occupational methods, uses, and durations for
1543 farmers versus lawncare professionals, etc.) and observed levels in urine as it is the characterization of
1544 exposure that is the greatest weakness of the epidemiological studies (Blair and Zahm 1990).
1545 Developing models helps researchers to understand differences in exposures across methods of
1546 applications, and assists in defining likely exposure routes for future studies. In this analysis, these data
1547 and models provide the context for the interpretation of potential cellular impacts as they relate to a
1548 potential mode of action for carcinogenic outcomes in humans.

1549 **6.1 Predictors of 2,4-D and MCPA Exposure in Occupational Settings and Observed Urinary**
1550 **Levels**

1551
1552 Table 10 provides a summary of biomonitoring studies from the literature and the conditions under
1553 which these urinary levels were measured. Most of the studies are in occupational settings, but several
1554 include spouses and family members. For example, Arbuckle et al. (2002) examined predictors of
1555 urinary 2,4-D levels among 126 farm applicators in the first 24 h after the first pesticide application of
1556 the season (Arbuckle et al. 2002). The variables pesticide formulation, protective clothing, application
1557 equipment, handling practice, and personal hygiene practice were found to explain 39% of the
1558 variability in 2,4-D dose. The mean and geometric mean urinary levels among 43 applicators reporting
1559 use of 2,4-D were 27.63 and 5.63 µg/l, respectively.

1560 A similar study conducted by Bhatti et al. (2010) found much higher mean and geometric mean urinary
1561 levels but this study followed noxious weed control applicators over a 12 week period (longer than the
1562 Arbuckle study). Overnight (approximately 12 h) urine samples were obtained from study participants
1563 every other week after a typical day of 2,4-D application. A total of 140 urine samples were collected (45
1564 samples were collected in 1994 and 95 samples were collected in 1995). The best-fit multivariate model
1565 explained only approximately 23% of the variation in predicted urinary levels.

1566 Harris et al. (2002) found that volume of pesticide applied explained 20% of the variation in 2,4-D dose
1567 among 98 professional turf applicators over a 1-week period (mean and geometric mean daily dose of
1568 2,4-D 1399 and 420 mg, respectively). Type of spray nozzle used and the use of gloves while spraying
1569 explained an additional 43% of variation in 2,4-D dose (Harris et al. 2002). In a study of 34 farm
1570 applicators and their families with urine samples collected 1 day before through 3 days after an
1571 application, glove use, repairing equipment, and number of acres treated were found to be the most
1572 significant predictors of 2,4-D concentration among applicators (geometric mean urinary 2,4-D

1573 concentration 1, 2, and 3 days after application was 33.4, 33.3, and 16.3 mg/g creatinine, respectively)
1574 (Alexander et al. 2007).

1575 In a study of Swedish forestry workers, Frank et al. (1985) measured urine levels for six volunteer
1576 workers involved in mixing and loading 2,4-D ester solutions into aircraft and in guiding the spray
1577 aircraft in two conifer release programs during 1981 and 1982. The highest measured urinary level (22.2
1578 $\mu\text{g}/\text{kg}$ body weight/day) was backcalculated to a maximum absorbed dose of 60 $\mu\text{g}/\text{kg}\text{-d}$ assuming an
1579 18-hr half life for excretion of 2,4-D.

1580 In a study involving 12 applicators spraying only 2,4-D, Figgs et al. (2000) collected 45 urine specimens
1581 over time with concentrations ranging from 1.0 to 1,700 (lg 2,4-D/g creatinine/L urine) that increased
1582 logarithmically as spraying time increased. However, the relationship between urine concentrations and
1583 potential exposures was not provided or explored.

1584 Lavy et al. (1987) evaluated potential exposures to US Forestry Service personnel occupationally
1585 exposed to 2,4-D under four different application regimes, including backpack spraying, injection bar,
1586 Hypohatchet, and hack-and-squirt. Four groups of 20 workers each were selected who had no known
1587 herbicide exposure for at least seven days before beginning the test. Each worker applied herbicide in a
1588 12-d, two-part test, including a preapplication day, an application day on which usual application
1589 procedures were used followed by four days of no new exposures. The following week, the workers had
1590 another preapplication day, a second application day on which special precautions to minimize exposure
1591 were taken followed by four days of no new exposure. The total urine excreted each day was collected
1592 from each worker. The authors measured an average of backpack applicators applying 2,4-D during a 7-
1593 h day in T-1 had an absorbed dose of 0.088 mg/kg. The average absorbed doses of 2,4-D during T-1 for
1594 others applying Tordon 101-R were 0.010, 0.085 and 0.029 mg/kg for the injection bar, Hypohatchet and
1595 hack-and-squirt crews, respectively.

1596 GM urinary 2,4-D levels for broadcast spray applicators in Thomas et al. (2010) (GM 21 µg/l, range 2.5–
1597 270 µg/l for day-1 urine samples) were lower than those measured by Acquavella et al. (2006) (GM 64
1598 µg/l, range 2–1856 mg/l), but higher than those reported by Arbuckle et al. (2002) (GM 5.4 µg/l, range
1599 0.5–410 µg/l) for 43 Ontario farm applicators.

1600 Durkin et al. (2004) developed a physiologically-based pharmacokinetic (PBPK) model of 2,4-D in
1601 humans based on an unpublished study by Dow Chemical involving rats. They then calibrated the model
1602 using human data from Sauerhoff et al. (1977) and Feldmann and Maibach (1974). The model considers
1603 flow-limited pH trapping modified to consider tissue binding, binding to plasma, and high-dose inhibition
1604 of urinary excretion in tissue, skin, GI tract, kidney, liver, and blood. Exposure is primarily through
1605 dermal contact. Lavy et al. (1987) measured exposures to backpack applicators in a study for the US
1606 Forest Service, and these data were used in the model to determine disposition of 2,4-D under typical
1607 exposure conditions.

1608 Thomas et al. (2010) monitored private pesticide applicators in the Agricultural Health Study (AHS)
1609 epidemiological cohort was monitored around the time of their agricultural use of 2,4-D and obtained
1610 urinary samples as well as patch, hand-wipe, and personal air samples. Pre-application urinary levels
1611 averaged approximately 8 µg/l, which increased to an average of 25 µg/l following several days of 2,4-D
1612 application.

1613 In an observational research study of 135 preschool children and their caregivers in NC and OH, Morgan
1614 et al. (2008) report measured urinary levels based on several spot samples. The highest measured
1615 sample in a child was 12.5 µg/l, translating to a dose of 0.28 mg/kg-d assuming a daily urine excretion of
1616 22.4 ml/kg bw for children (Morgan et al. 2008), a value 35 times lower than the IRIS RfD of 0.01 mg/kg-
1617 d.

1618 Aylward et al. (2010) report the 50th and 95th percentiles from the National Health and Nutrition
1619 Examination Survey (NHANES) dataset, which shows levels in the general public based on several spot
1620 samples that are comparable to Morgan et al. (2008).

1621 6.2 Exposure Pathways

1622 The available data suggest that the primary exposure pathway for residential and non-occupational
1623 exposures is dermal exposure following application (e.g., treatment of yards, etc.) followed by oral
1624 exposure and that inhalation exposures are negligible (Harris et al. 1992; Health Canada 2005; 2006)
1625 representing less than 0.2% of overall exposures in occupationally-exposed adults (Munro 1992; Durkin
1626 et al. 2004). The highest inhalation exposures have been documented for workers in production facilities
1627 (Knopp 1994); even sprayers do not experience significant inhalation exposures (Durkin et al. 2004;
1628 Munro 1992). Consequently, the primary exposure to 2,4-D and MCPA in the environment is dermal
1629 and, to a lesser extent, oral ingestion. Studies summarized in Ross et al. (2005) show that less than 10%
1630 of dermally applied 2,4-D is absorbed in occupationally exposed adults.

1631 In risk assessments developed to support reregistration of MCPA in Canada, Health Canada (2006)
1632 estimates the contribution of inhalation exposure to the overall exposure in postapplication scenarios as
1633 negligible, due to the dilution effect of outdoor use and considering the study by Yeary and Leonard
1634 (1993) wherein MCPA was not detected in the breathing zone of 25 applicators during the application of
1635 MCPA to residential lawns, trees and shrubs (limit of detection of 0.001 mg/m³). Similarly, inhalation has
1636 been shown to contribute less than 2% of the cumulative exposure among 2,4-D applicators (Grover et
1637 al. 1986). Further, air concentrations of up to 20 mg/m³ did not correspond with measurable exposure in
1638 any of the bystanders to a 2,4-D spray application (Harris et al. 1992).

1639 A series of studies by Nishioka et al. (1996; 1999; 2001) evaluating transport of lawn-applied 2,4-D into
1640 homes, including measurements of how much was tracked relative to how much was applied, and the

1641 primary tracking mechanisms found low but measurable concentrations of 2,4-D inside homes and
1642 conclude that although low, these concentrations could lead to dermal and oral (but not inhalation)
1643 exposures. Similarly, Mustonen et al. (1986) measured air breathing space of workers and found very
1644 low air concentrations from spraying concurrent with measured urinary concentrations in 19 workers
1645 and conclude that dermal exposures represent the primary source of exposures.

1646 The exception to the dermal pathway as the dominant exposure pathway is for children. Wilson et al.
1647 (2010) in a study of children exposed to residential use of pesticides in North Carolina and Ohio found
1648 that the diet represented approximately 80 – 90% of the daily dose of 2,4-D for children. Inhalation was
1649 3 - 4% and dermal 9 – 15%. Based on observed urinary levels in 287 children, the aggregate potential
1650 dose was approximately 9 - 10 ng/kd-d (for reference, the IRIS RfD is 0.01 mg/kg-day or 10^4 ng/kd-d).
1651 The maximum predicted aggregate dose ranged from 98 to 177 ng/kg-d.

1652 6.3 Biomonitoring Equivalents

1653 Biomonitoring equivalents are urine and/or blood concentrations associated with exposures in humans
1654 to chemical-specific regulatory standards such as the RfD.

1655 Aylward et al. (2010) reviewed the available biomonitoring data for 2,4-D from the United States and
1656 Canada and compared these data with expected biomonitoring equivalents based on regulatory
1657 threshold values to draw conclusions regarding the margin of safety for 2,4-D exposures based on
1658 published biomonitoring data for the general population, farm applicators, and farm family members.
1659 Aylward and Hayes (2008) estimated a biomonitoring equivalent in urine of 200 $\mu\text{g/L}$ (or 300 $\mu\text{g/g}$
1660 creatinine) associated with chronic, low-level exposure to 0.005 mg/kg-d. The analysis reflects oral
1661 exposures only – that is, 200 $\mu\text{g/L}$ in urine is the concentration associated with a daily, steady-state oral
1662 exposure of 0.005 mg/kg-d based on the following equation:

1663 $Urinary\ Level = \frac{Dose * BW}{V_{24hr}}$ (Eq.1)

1664 where:

1665 Urinary level = volume-based urinary level in µg/L

1666 Dose = Dose (RfD, or from Eq. 2)

1667 BW = body weight

1668 V_{24hr} = volume of urine in 24-hrs in l

1669 Given the potentially relevant positive results from the suite of 500 ToxCast assays described previously,
1670 these *in vitro* results were explored in the context of *in vivo* exposures using the following methodology.

1671 First, oral equivalent doses associated with the lowest biologically-relevant *in vitro* ToxCast assay results

1672 (Table 5) are estimated based on the following equation (Wetmore et al. 2011; Rotroff et al. 2010):

1673 $Dose = Assay * \frac{1 \frac{mg}{kg} \cdot d}{C_{ss}}$ (Eq. 2)

1674 where:

1675 Dose = oral equivalent dose in mg/kg-d

1676 Assay = lowest effective concentration for a biologically relevant pathway from the ToxCast assay in µM

1677 C_{ss} = steady-state concentration from PBPK model assuming 1 mg/kg-d oral exposure (Wetmore et al.

1678 2011; Durkin et al. 2004)

1679 The resulting predicted urinary level is based on the relationship provided in Aylward et al. (2008) and

1680 shown in Eq. 1. Table 11 show the results for children 4-12, adolescents up to 18, men and women

1681 including input assumptions for each, and Figure 7 provides a graphical depiction of these results in the
1682 context of the biomonitoring data.

1683 The highest predicted steady-state concentration for 2,4-D from the PBPK models is approximately 90
1684 μM , and the lowest biologically relevant ToxCast assay result is 1.5 μM based on cell growth kinetics. ,
1685 The associated estimated urinary levels are provided in the last column of Table 11 based on Eq. 1. The
1686 average C_{ss} (steady state body burden associated with 1 mg/kg exposure) predicted by Wetmore et al.
1687 (2011) is approximately 40 μM . The resulting ranges of predicted 2,4-D urinary levels associated with
1688 the lowest observed response from the *in vitro* ToxCast assays is 600 – 1250 $\mu\text{g/L}$ for children, 440 – 900
1689 $\mu\text{g/L}$ for adolescents, 470 to 960 $\mu\text{g/L}$ for women, and 560 to 1200 $\mu\text{g/L}$ for men. These compare to the
1690 biomonitoring equivalents developed by Aylward and Hays (2010) and Aylward (2008) of 200 $\mu\text{g/L}$ for an
1691 adult population based on a urinary level associated with exposure to the RfD.

1692 By parameterizing lognormal distributions using the parameters in Table 10 (geometric mean and
1693 geometric standard deviation, in most cases), 10th and 90th percentiles for each distribution were
1694 developed using the Crystal Ball Excel add-in and these are the basis of the whiskers in Figure 7.

1695 Comparing the backcalculated urine levels to the biomonitoring data from Table 10 shows that the only
1696 overlap between the levels associated with the potential for *in vitro* effects (at the lowest biologically
1697 relevant assay result) and data from biomonitoring studies is for one study in manufacturing workers
1698 (Knopp et al. 1994). The remaining studies show that even the predicted 90th percentiles fall well below
1699 these backcalculated levels with only a few exceptions for applicators. Most values even fall below the
1700 200 $\mu\text{g/L}$ level based on the RfD backcalculation. The data suggest some transient occupational
1701 exposures may come close to overlapping the backcalculated assay results, but the use of protective
1702 gear would preclude these exposures from occurring.

1703 There is less data available for MCPA, but based on the results presented in Wetmore et al. (2011)
1704 combined with Eq. 1 (Aylward et al. 2008) shows that backcalculated urinary levels are approximately
1705 450 µg/L for children, 320 µg/L for adolescents, 240 µg/L for women, and 310 µg/L for men. Using the
1706 published IRIS RfD of 0.0005 mg/kg-d results in predicted urinary levels an order of magnitude lower,
1707 and the regulatory value used by Health Canada and US EPA Office of Pesticide Programs (0.0044 mg/kg-
1708 d) falls in-between. There are no direct MCPA biomonitoring data available, but Figure 7 shows these
1709 backcalculated values in the context of the Arbuckle et al. (2006) study (for which 2,4-D and MCPA
1710 urinary levels co-eluted). The backcalculated bioassay results show no overlap, but the backcalculated
1711 level from the RfD falls within the biomonitoring data for the applicators. However, there is no
1712 particular relevance of the RfD with respect to potential carcinogenicity.

1713 6.4 Summary of Exposure and Biomonitoring

- 1714 • Dermal absorption represents the primary exposure route in both occupationally-exposed
1715 individuals and the general public, followed by ingestion, while inhalation exposures are
1716 negligible in residential settings and largely negligible even in occupational settings
- 1717 • There are numerous studies available for characterizing 2,4-D and MCPA concentrations in
1718 homes following residential application of 2,4-D
- 1719 • Occupational exposures depend heavily on the amount of protective clothing that is worn and
1720 vary widely; exposures at the 95th percentile in the general public are typically less than 100
1721 times the IRIS RfD of 0.01 mg/kg-d
- 1722 • Backcalculated urinary levels using the results from the lowest observed bioassay result
1723 required to alter a relevant biological pathway *in vitro* are an order of magnitude higher than
1724 levels based on the RfD for MCPA, and a factor of five higher for 2,4-D
- 1725 • There are orders of magnitude difference between estimated urine levels equivalent to the
1726 lowest ToxCast concentrations required to alter biologically relevant pathways and

1727 biomonitoring data. The difference is less for occupational exposures, but still generally large,
1728 even at the 90th percentile

1729 **7.0 Discussion and Conclusions**

1730 Chlorophenoxy compounds have been in use since the 1940s, and despite numerous regulatory and
1731 non-regulatory reviews, they continue to be controversial, particularly with respect to carcinogenic
1732 outcomes. Early epidemiologic studies that defined exposures in terms of job matrices rather than
1733 through quantitative estimates of actual exposures to chlorophenoxy compounds found some
1734 associations with various lymphomas, particularly NHL. The Swedish studies, in particular, (Eriksson et al.
1735 2008) found significant associations between exposure specifically to MCPA and NHL, and STS (Hardell
1736 and Bengtsson 1983) although those associations were not confirmed in other studies. Associations
1737 were limited to case-control studies with small sample sizes that were not confirmed by the cohort
1738 studies. Potential associations in case-control studies were based on univariate analyses without
1739 including other potential exposures and/or known risk factors, while those studies incorporating the
1740 variety of exposures experienced in the environment generally show no statistically significant role for
1741 exposures to chlorophenoxy compounds. More recent epidemiologic studies linking genetic markers of
1742 effect (e.g., t(14;18) translocations) find no association with exposure to chlorophenoxy compounds and
1743 carcinogenic outcomes. Genomic instability observed in agricultural workers has not been associated
1744 with exposure to 2,4-D, and in any event have been shown to be transient and reversible (Garry et al.
1745 2001). Short-term immunosuppressive effects have been observed in humans (Faustini et al. 1996)
1746 following exposure to 2,4-D and MCPA, and although most of these effects were transient, some were
1747 still observed 70 days after exposure. Given that exposures to 2,4-D and MCPA are episodic in nature,
1748 the question arises as to what role repeated short-term immunosuppression might play in contributing
1749 to an increased risk of developing NHL.

1750 Toxicological studies conducted *in vivo* using traditional rodent assays over one or two years showed no
1751 treatment-related carcinogenic effects although other effects were noted, particularly to the kidney and
1752 liver. Animal studies *in vitro* are equivocal, with some suggesting that 2,4-D and/or MCPA are able to
1753 cause chromosomal aberrations and interrupt key cellular functions while others do not (summarized in
1754 Table 9), but generally showing effects at concentrations exceeding renal transport mechanisms. Studies
1755 involving human cell cultures, or human cells derived from *in vivo* exposures do suggest that 2,4-D
1756 and/or MCPA are capable of causing chromosomal aberrations in some studies (Korte and Jalal 1982;
1757 Arias 2003; Gonzalez 2005; Maire 2007) but not in others (Charles 1999a; Linnainmaa 1984), and both
1758 2,4-D and MCPA showed negative results across a battery of ToxCat genotoxicity assays (Knight et al.
1759 2009). Interestingly, some positive associations are noted for exposure to a commercial product
1760 containing 2,4-D but not for the pure 2,4-D acid or salt (Mustonen et al. 1986; Holland et al. 2002).

1761 Studies are quite consistent, however, in suggesting that exposure to 2,4-D can disrupt other cellular
1762 functions and lead to cell replication necessary for tumor promotion *in vivo*, but only after longer
1763 exposure periods and only at the highest concentrations tested. Observed 2,4-D toxicity generally
1764 occurs at doses above renal saturation, i.e., doses above which excretory processes could readily
1765 eliminate the chemical. Nonetheless, an extensive review of 2,4-D by the German Research Foundation
1766 (Henschler and Greim 1998, p. 90) concluded there was sufficient evidence of a weak promoting effect
1767 of herbicide formulations of 2,4-D. Studies in human volunteers have shown that while immunological
1768 effects following exposure were observed (Faustini et al. 1996), effects only persisted during exposure.

1769 Although effects showed a relationship to urine levels of 2,4-D, the trend in the relationship was not
1770 statistically significant. Thus, the combined evidence indicates that it is only at exposures exceeding
1771 renal transport mechanisms that effects are observed. What is less well understood is whether
1772 exposures at lower concentrations but over longer periods of time might be sufficient to effect relevant

1773 cellular changes. The episodic nature of environmental exposures, however, suggests this is unlikely to
1774 occur.

1775 The etiology of NHL suggests a multi-stage process including specific chromosomal translocations
1776 present in nearly 90% of all cases coupled with proliferating events such as interruption of apoptosis,
1777 increased chronic inflammation as a result of oxidative stress or peroxisome proliferation, increased
1778 production of free radicals, or direct proliferation of a mutation. The evidence does not support an
1779 association between exposures to 2,4-D and/or MCPA and direct DNA interaction; however, the specific
1780 chromosomal translocations observed in NHL (e.g., t(14;18) are prevalent in healthy individuals
1781 (Limpens et al. 1995); therefore, it is not unreasonable to assume that a significant proportion of
1782 individuals exposed to 2,4-D and/or MCPA already have the required translocations.

1783 NHL most often arises in premature and/or naïve B-cells, and a host of genetic markers have been
1784 identified with respect to key subclinical features of the disease. Epidemiologic studies are
1785 incorporating these genetic markers, and these studies show no association between exposure to
1786 chlorophenoxy compounds broadly defined (of which 2,4-D is likely to be a predominant exposure) and
1787 these markers. Although the toxicological data in human cell cultures and/or *in vivo* human exposures
1788 suggest that there are plausible mechanisms by which exposure to 2,4-D could promote NHL by causing
1789 cellular proliferation as evidenced by positive responses in replicative index assays, the doses at which
1790 these effects are noted are well above levels observed even in occupational settings (based on
1791 measured urine) and are likely to exceed saturation of renal transport mechanisms.

1792 Exposure data and measured urine levels in workers and in the general public show that exposures to
1793 2,4-D and MCPA in the environment are at levels below RfDs published in IRIS or by the Office of
1794 Pesticide Programs. Modeled urine levels based on chronic exposures to the RfD for 2,4-D, when
1795 compared to biomonitoring data, show that urine levels, even in occupationally-exposed individuals, are

1796 below levels of concern. Exposures in the environment are predominantly through dermal absorption,
1797 and studies show less than 10% of 2,4-D and MCPA are dermally absorbed. Studies that have estimated
1798 daily doses to the general public based on repeated measurements in the home show these doses are
1799 orders of magnitude below the IRIS RfDs.

1800 The only plausible potential for risk that can be hypothesized would be in an occupationally exposed
1801 sub-population with specific polymorphisms, family history, and/or lifestyle characteristics identified as
1802 risk factors for NHL; but that assumes that occupational exposures would be high enough, and sustained
1803 enough, to lead to adverse effects. Figure 7 shows the difference between measured urine levels and
1804 estimated urine levels from exposures at the RfD or exposures at the lowest biologically relevant
1805 ToxCast assay results. As seen in the Figure, the difference is significant at the 90th percentile across all
1806 but a few occupational studies. This difference translates to several orders of magnitude for the general
1807 public, and at least an order of magnitude, in general, for occupational exposures. Given that the RfD is
1808 a dose associated with no effects (including a margin of safety), and that *in vitro* bioassay results may
1809 not translate to *in vivo* effects, the combined evidence indicates it is highly implausible that exposure to
1810 2,4-D and/or MCPA are associated with a risk of developing NHL or other lymphohematopoietic cancers.

1811 However, exposures and potential impacts are considered only for exposure to 2,4-D and MCPA in
1812 isolation. From a cumulative risk perspective, there are likely numerous concurrent exposures, some of
1813 which exert similar immunosuppressive or proliferative effects, and the combined impact of these
1814 exposures has not been considered. That represents a more complex question that cannot be
1815 addressed by the data in-hand since so much of that is unique to the individual, but there is an
1816 opportunity going forward for epidemiologic and other studies to evaluate these combined exposures
1817 more comprehensively. That said, given the difference between observed exposures as measured by

1818 urine levels and estimated urine levels from regulatory or ToxCast values indicates that environmental
1819 exposures to 2,4-D and/or MCPA are unlikely to be risk drivers even in a cumulative risk context.

1820 **Conflict of Interest**

1821 Funding support was provided by the Environmental Health Research Foundation (EHRF), a nonprofit,
1822 nonpartisan scientific research foundation to E Risk Sciences, a private consulting company specializing
1823 in developing risk-based tools and analyses to support environmental decision making with both private
1824 and public clients. I declare no competing financial interests or other conflicts of interest.

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