Differences in the Lung Tumor Incidence and Survival Rate for CD-1 Mice Between 78-Week and 104-Week Studies in the Historical Control Data

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ABSTRACT

In 2010 and 2012 the California Department of Pesticide Regulation completed human health risk assessments for the pesticide chloropicrin that included an evaluation of its carcinogenic potential. Chloropicrin is genotoxic in vitro and caused tumors in rodent oral and inhalation bioassays. A quantitative cancer analysis was performed based on an 18-month inhalation study that showed statistically elevated incidence in lung tumors in female CD-1 mice. In the weight of evidence for carcinogenicity, there was some uncertainty because the combined incidence of adenomas and carcinomas in the females (22/49 or 45%) was only marginally statistically significant, partly due to the high incidence in the control group (13/48 or 27%, all adenomas). It was suggested that the tumor incidences at the high dose could be higher had the study duration been longer (e.g., 2 years). At the time, the only available historical control data for CD-1 mice was from studies performed between 1987 and 1996. Since then, two new reports were published covering more recent studies. The lung tumor incidences and survival rate in the 78-week and 104-week studies from these 3 historical reports (all from Charles River) were compared. In the 78-week studies, the range of pulmonary adenomas and carcinomas in female CD-1 mice was 0-16.0% and 0-12.0%, respectively; the combined incidence was 1.7-25.7%. In the 104-week studies (24 studies) the range was 0-26.7% for adenomas and 0-20.0% for carcinomas; the range for the combined incidence was 3.9-35%. The incidence of pulmonary adenomas in the control group in the chloropicrin 18-month study was clearly outside the historical range for the 78-week studies and even outside the historical range for the combined incidence. The historical control range increased with the longer studies, mostly due to increases in adenomas. The survival rate of control females ranged from 63.3-83.3% in the 78-week studies and 18.6-55.4% in the 104-week studies. While survival rate decreased from 78 week to 104 week, it was above the 25% cutoff recommended by USEPA guidelines, except for one study. Therefore, it should be possible to conduct a 2-year oncogenicity study using CD-1 mice. However, health issues at the end of life expectancy including increased incidence of tumors in the control may compromise the overall interpretation of the tumor data. Use of a different strain with greater longevity (B6C3F1 mice) may be a way to overcome survival/health issues.

INTRODUCTION

The California Department of Pesticide Regulation (CDPR) completed two risk assessments for chloropicrin. The risk assessment completed in 2010 addressed ambient air and bystander (drift) exposures and was used to determine whether or not to list chloropicrin as a toxic air contaminant (Lewis, 2010). The 2012 RCD addressed occupational exposure. In an 78-week inhalation study for chloropicrin, female mice had an increase in pulmonary adenomas and carcinomas at the high dose that was marginally significant by Fisher's exact test (p = 0.053), but clearly significant by Poly-3 trend test (p = 0.03) (Table 1). In both RCDs, the weight of evidence for carcinogenicity concluded that there was sufficient evidence of carcinogenicity to conduct a quantitative assessment of carcinogenicity. Table 2 summarizes the weight of evidence supporting this conclusion. In the risk appraisal in both documents, there was a discussion of possible reasons why the increase in tumors was not greater. One confounding factor was the high incidence of adenomas in the concurrent control group. The incidence appeared to be outside the historical control range based on a report by Charles River (Giknis and Clifford, 2000). Other considerations were that the study was only 78 weeks long and the incidence might have been higher in a longer study. There was discussion about whether a longer term mouse oncogenicity study should be requested to determine if the increase in lung tumors in female mice would be more pronounced with longer exposure. One question that arose in this discussion was whether a sufficient number of CD-1 mice would survive to the end of a 2-year study to meet guidelines (> 25% survival needed at study termination). To answer this question the historical control data for Charles River CD-1 mice was evaluated.

Table 1. Neoplastic Microscopic Lesions in the Lungs of Female Mice Exposed to Chloropicrin Vapors for 78 Weeks[a]

	Treatment Level (ppm)			
Pulmonary Lesion	**0**	**0.1**	**0.5**	**1.0**
Adenoma	13/48[b] (27%)	9/48 (19%)	17/47 (36%)	19/49 (39%)
Carcinoma	0/48[b] (0%)	4/48 (8%)	3/47 (6%)	4/49 (8%)
Combined Adenoma and Carcinoma	13/48[c,+b] (27%)	12/48 (25%)	20/47 (43%)	22/49 (45%)
Combined Adenoma and Carcinoma - Adjusted	13/42[d,+d] (31%)	12/41 (29%)	20/43 (46%)	22/42* (54%)

a Burleigh-Flayer *et al*., 1995

b The denominator is the number of animals at risk which are animals that survived up to the day the first tumor was observed, 253 days.

c Historical control data published by Charles River for this strain for studies conducted between 1987 and 1996 had an average incidence of 8.7% (0-27%) for adenomas in females (44 studies) (Giknis and Clifford, 2000).

d The animals at risk was determined in the Poly-3 trend test by weighting the animals without tumors based on their time of death. The Poly-3 trend test is utilized by the National Toxicology Program (Portier and Bailer, 1989).

+,+b Significant trend at p < 0.05 and 0.01 and 0.001, respectively, based on either the Armitage-Cochran trend test (Gart *et al*., 1986) or the Poly-3 trend test.

* Significantly different from the control group based on pair-wise comparison test within the Poly-3 trend test at p < 0.05.

Table 2. Evidence Supporting a Quantitative Assessment of Carcinogenicity for Chloropicrin

1. Chloropicrin is a strong electrophile
2. Chloropicrin was positive in all 8 reverse mutation assays with *Salmonella*[1-8]
   a. In 6 of 8 studies, the positive responses were seen with TA100 strain with activation
3. Two *in vitro* tests for clastogenicity were positive
   a. Chromosomal aberrations assay with CHO cells[9]
   b. Sister chromatid exchange assay with human lymphocytes[10]
4. Chloropicrin tested positive in three in vitro tests for DNA damage
   a. SOS chromotest with *E. coli*[11]
   b. SCGE assay with Chinese hamster ovary (CHO) cells[12]
   c. Comet assay with TK6 cells[13]
5. Female mice exposed to chloropicrin vapors for 78 weeks had an increase in pulmonary adenomas and carcinomas[14]
   a. Combined incidence was significant by trend analysis (p < 0.01) and by pair-wise comparison at the high-dose (p < 0.05), when adjusted for survival
   b. Incidence of adenomas at the high dose (37%) was clearly outside the historical control range reported by the supplier (0-27%) during a similar time period[15]
   c. Increase in the multiplicity of these tumors was significant by trend analysis
   d. There was a slight reduction in time to tumors at the high dose
   e. Tumor incidence might have been higher if:
      1) Study duration were 104 weeks rather than 78 weeks
      2) Dose levels were higher
      3) Body weights and caloric intake were not reduced
6. Female rats administered chloropicrin daily for 2 years by oral gavage had an increase mammary fibroadenomas[16]
   a. Increase was significant by trend analysis (p < 0.05) and by pair-wise comparison at the high dose (p < 0.05)

1. Shirasu *et al*., 1982; 2. Haworth *et al*., 1983; 3. Moriya *et al*., 1983; 4. Kawai *et al*., 1987; 5. San and Wagner, 1990; 6. Sariaslani and Stahl, 1990; 7. Giller *et al*., 1995; 8. Schneider *et al*., 1999; 9. Putman and Morris, 1990; 10. Garry *et al*., 1990; 11. Giller *et al*., 1995; 12. Plewa *et al*., 2004; 13. Liviac *et al*., 2009; 14. Burleigh-Flayer *et al*., 1995; 15. Giknis and Clifford, 2000; 16. Stauter, 1995.

METHODS

Charles River has published three reports summarizing the historical control data for their outbred CD-1 mouse strain, Crl:CD1(ICR), from studies initiated between 1987 and 2006 (Giknis and Clifford, 2000, 2005, 2010). Data from 54 studies initiated between 1987 and 2000 in 11 different laboratories were reported by Giknis and Clifford (2000, 2005). Mice used in these studies came from 4 different Charles River breeding sites. Data from another 14 studies initiated between 2002 and 2006 conducted in 5 different laboratories were later reported (Giknis and Clifford, 2010). This report did not indicate which Charles River breeding facilities the mice came from.. These reports contained individual study tumor incidence in control males and females, but they did not identify the conducting laboratory or Charles River breeding site for each study so inter-laboratory and inter-breeding site variability could not be analyzed. In these reports, if no tumors were seen the incidence was left blank. This resulted in some uncertainty especially when neither pulmonary adenomas or carcinomas were observed. Since these pulmonary tumors are fairly common, it seems unlikely that neither of these tumors were seen in any of the control females. Therefore, five of the 104-week studies were excluded from the analysis because they reported neither of these lung tumors. In the final analysis of the lung tumor data, 63 studies were included with 33 studies that were 78 weeks in duration, 6 studies that were 91-100 weeks in duration and 24 studies that were 104-weeks in duration. Percent survival at study termination was also available in these reports, but only 37 studies provided survival data (15, 5 and 17 studies that were 78, 91-100 and 104 weeks in duration, respectively). Median, 25th and 75th percentiles and range for lung tumor incidence and survival rate were calculated for studies based on study duration initially. These data were further analyzed to see if the tumor incidence or survival rate was different between the first and second decade between 1987 and 2006. Differences in tumor incidence and survival rate were expected with studies of different durations, so no statistical analysis was performed on these differences. To determine if the tumor incidence or survival rate among studies of the same duration were different between the two decades, T-tests assuming unequal variation were performed.

RESULTS AND DISCUSSION

The individual study incidence of pulmonary adenomas and carcinomas in female CD-1 mice is summarized in **Figure 1** grouped by study duration. The difference in incidence between the 78-wk studies and 104-wk studies can be seen visually in the increase in adenomas. The increase in carcinomas in the longer studies was more sporadic. Generally, the incidence of adenomas was greater than the carcinomas in any study, but there were a few studies that had higher incidences of carcinomas than adenomas, including a few studies that only reported carcinomas. **Figure 2** and **Table 3** summarize the median, 25th and 75 percentiles and range for adenomas, carcinomas and combined tumors for the 78-wk, 91-100-wk and 104-wk studies. The variation in the tumor incidence between studies even with the same study duration was large which can be seen visually in the Figure 1 and numerically based on the large ranges and differences in the 25th and 75th percentiles in Table 3.

There appeared to be an increase in the pulmonary tumor incidence in the more recent studies when they were separated into two decades as shown in **Figure 3**. However, unequal distribution of 78-wk and 104-wk studies between the two decades may have contributed to this so the incidence of tumors was broken down further by decade and study duration (**Figure 4**). Among the 78-wk studies, the incidence of both adenomas and carcinomas appear to have decreased rather than increased in the second decade, however, the differences were not statistically significant. This difference may just be an artifact of the small number of 78-wk studies conducted in the second decade. With 104-wk studies, the incidence of both the adenomas and carcinomas appear to have increased in the second decade, but this difference was also not statistically significant even though there were an equal number of studies in both decades. The most likely cause of the large variation in tumor incidence is the numerous conducting laboratories. Other possible explanations for this difference are 1) inter-laboratory variability, 2) inter-breeding site variability and 3) genetic drift. Genetic drift is more likely to occur in outbred strains such as the Charles River CD-1 mouse strain than inbred strains (Kuroiwa *et al*., 2013).

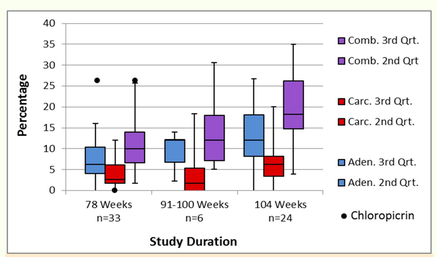

Figure 2. Change in Lung Tumor Incidence in Female CD-1 Mice with Study Duration

Table 3. Median, Percentiles and Range of Pulmonary Adenomas and Carcinomas in Female CD-1 Mice Based on Study Duration

		78-Wks	91-100 Wks±	104 Wks
	n	33	6	24
Adenomas (%)	Median	6.3	12.0	12.0
	25th Percentile	4.0	6.8	8.2
	75th Percentile	10.4	12.2	18.1
	Range	0.0 – 16.0	2.2 – 14.0	0.0 - 26.7
Carcinomas (%)	Median	2.7	1.7	6.2
	25th Percentile	1.7	0.0	3.5
	75th Percentile	6.1	5.3	8.2
	Range	0.0 – 12.0	0.0 - 18.4	0.0 – 20.0
Combined (%)	Median	10.0	12.0	18.2
	25th Percentile	6.7	7.2	14.8
	75th Percentile	14.0	18.0	26.2
	Range	1.7 - 25.7	5.1 - 30.6	3.9 – 35.0

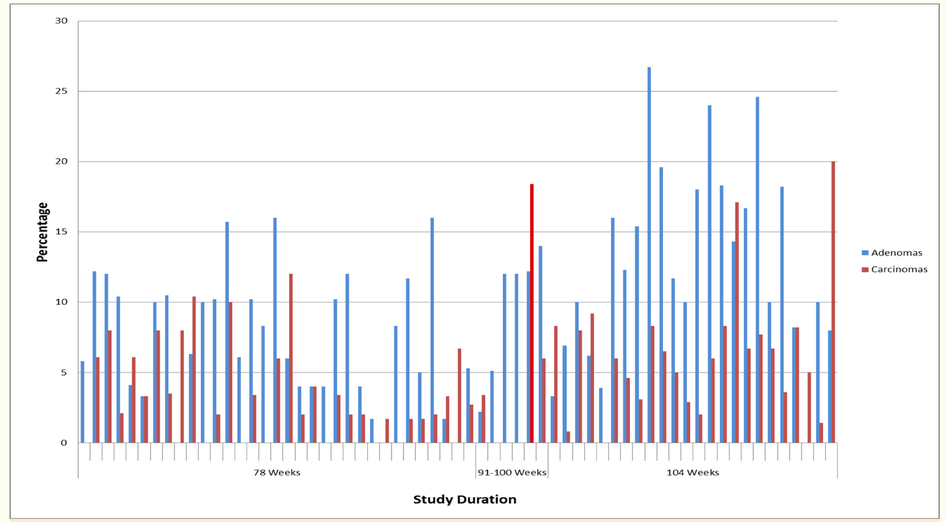

Figure 1. Individual Study Lung Tumor Incidence in Control Female CD-1 Mice Grouped by Study Duration

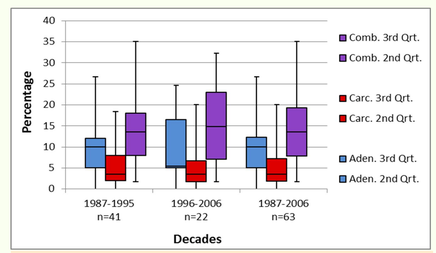

Figure 3. Change in Lung Tumor Incidence in Female CD-1 Mice Over Decades

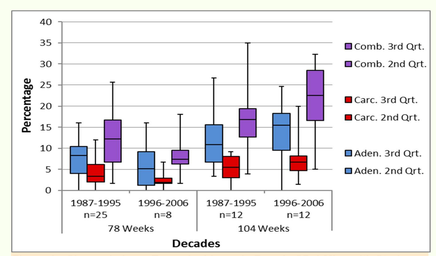

Figure 4. Change in Lung Tumor Incidence in Female CD-1 Mice with Study Duration Over Decades

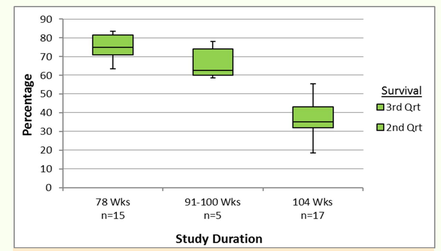

Figure 5. Differences in Survival of CD-1 Mice Based on Study Duration

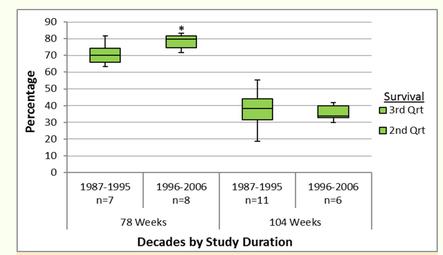

Figure 6. Differences in Survival of Female CD-1 Mice over Two Decades Based on Study Duration

Table 4. Median, Percentiles and Range of Survival Rates in Female CD-1 Mice

		78 Wks	91-100 Wks	104 Wks
	n	15	5	17
Survival (%)	Median	75.0	62.7	35.0
	25th Percentile	70.8	60.0	32.0
	75th Percentile	81.5	74.0	43.1
	Range	63.3 - 83.3	58.7 – 78.0	18.6 - 55.4

When the survival data was analyzed based on study duration, the percentage of animals surviving to study termination diminished as study duration increased which is expected (**Table 4**, **Figure 5)**. Unlike the lung tumor incidence the variation in survival was not as great when studies were separated by study duration, as can be seen by the range and small differences in the 25th and 75th percentiles. If a 2-year oncogenicity study is to be conducted, it is critical that a sufficient number of animals (25%) survive to the study termination. Among the 104-wk studies which reported survival data, the median survival rate was 35.0%. The range was 18.6% to 55.4% so there is a slight risk that the survival rate will drop below the recommended 25%.

The survival data was also analyzed to determine if there was any change in the survival rate between the two decades over which these studies were conducted. **Figure 6** shows the median, the 2nd and 3rd quartiles and range for survival grouped by decades as well as study duration. The increase in survival seen in the 78-wk studies from the recent decade was statistically significant by t-test assuming an unequal variation (p = 0.013). It seems unlikely that this increase is due to genetic drift in that it was not seen in the 104-wk studies, too. More likely these differences were due to 1) inter-laboratory variability, 2) inter-breeding site variability, or 3) chance.

CONCLUSIONS

- The combined incidence of pulmonary adenomas and carcinomas in female control group of the chloropicrin oncogenicity study with CD-1 mice is clearly outside the historical control range for 78-wk studies conducted over two decades. This is especially noteworthy given the large variability in the historical control data.
- The increase in tumors from 78-wk studies to 104-wk studies was primarily due to an increase in adenomas.
- The lung tumor incidence appears to be increasing in the more recent 104-wk studies. The cause is uncertain, but some possible explanations are 1) inter-laboratory variability, 2) inter-breeding site variability, or 3) genetic drift.
- The median survival in 104-wk studies was 35.0% with a range of 18.6 to 55.4%. This data shows that it is possible to conduct a 2-year study with CD-1 mice, but there is a slight risk of not having 25% of controls surviving to the study termination.
- The median survival rate in the more recent 78-wk studies was significantly higher, but this was not seen in the 104-wk studies. It seems less likely that genetic drift was the cause, but inter-laboratory and inter-breeding site variability may have contributed.
- Use of a different strain with a greater longevity such as B6C3F1 mice could eliminate the survival concern.

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