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Flawed analysis of an intentional human dosing study and its impact on chlorpyrifos risk assessments

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ABSTRACT

In March 1972, Frederick Coulston and colleagues at the Albany Medical College reported results of an intentional chlorpyrifos dosing study to the study's sponsor, Dow Chemical Company. Their report concluded that 0.03 mg/kg-day was the chronic no-observed-adverse-effect-level (NOAEL) for chlorpyrifos in humans. We demonstrate here that a proper analysis by the original statistical method should have found a lower NOAEL (0.014 mg/kg-day), and that use of statistical methods first available in 1982 would have shown that even the lowest dose in the study had a significant treatment effect. The original analysis, conducted by Dow-employed statisticians, did not undergo formal peer review; nevertheless, EPA cited the Coulston study as credible research and kept its reported NOAEL as a point of departure for risk assessments throughout much of the 1980's and 1990's. During that period, EPA allowed chlorpyrifos to be registered for multiple residential uses that were later cancelled to reduce potential health impacts to children and infants. Had appropriate analyses been employed in the evaluation of this study, it is likely that many of those registered uses of chlorpyrifos would not have been authorized by EPA. This work demonstrates that reliance by pesticide regulators on research results that have not been properly peer-reviewed may needlessly endanger the public.

1. Introduction

The organophosphorus insecticide chlorpyrifos has become one of the most controversial pesticides in current use in the United States (PANNA v EPA 2015; 2017; USEPA 2017; LULAC v Wheeler 2018). Past registrations for chlorpyrifos permitted residential uses including structural pest control and nuisance pest control in the home and garden. In recent decades, actions taken in response to public health concerns have restricted its use almost exclusively to agriculture (USEPA 1997a; 2006). Chlorpyrifos exposure can result in acute poisoning as well as long-term developmental effects in humans (2011, 2015; Roberts and Reigart, 2013; Rauh et al., 2006; Rauh, 2018; Hertz-Picciotto et al., 2018). The U.S. Environmental Protection Agency (USEPA) issued a hazard evaluation report in 2016 recommending that all remaining registrations for agricultural use be eliminated (USEPA 2016), but the Agency Administrator rejected this report on March 29, 2017 and supported continued registrations through at least 2022 (USEPA 2017).

EPA risk assessments are generally based on scientific studies provided by pesticide registrants. These studies undergo internal review at EPA, but are not necessarily subject to outside peer review. One recent independent review criticized an industry-funded study of chlorpyrifos toxicity in rodents, finding the methods inappropriate and the conclusions misleading (Mie and Rudén, 2018). We explore the role of a registrant-sponsored intentional human dosing study that served as the basis for EPA's chlorpyrifos risk assessments for at least 15 years. Coulston and colleagues provided their conclusions from that study to Dow Chemical Company in a report titled "Safety Evaluation of DOWCO 179 in Human Volunteers." (Coulston et al., 1972). Their report concluded that the chronic no-observed-adverse-effect-level (NOAEL) was 0.03 mg/kg-day. EPA later adopted the study's findings without completing a thorough evaluation of the study design and statistical analysis. Our re-analysis demonstrates that the NOAEL reported in the study was incorrect.

The central questions we ask are: Why did a study so critical to risk assessment and protection of the public health never receive a rigorous review? What were the consequences of this failure to review? Are there safeguards in place to assure that evidence used in risk assessments is produced and reported in accordance with accepted scientific procedures?

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2. Reanalysis of the Coulston study

We have reanalyzed the Coulston Study data using both the original statistical method and modern computational tools that were not available at the time of the study. Our analysis has revealed two major flaws, as discussed in detail below: 1) the design of the study reduced its power to discern a treatment effect; and 2) the researchers' omission of valid data obscured an effect that their method would otherwise have identified. Examination of the data by both original and modern methods finds a treatment effect at levels below the dose identified by Coulston and colleagues as the NOAEL.

In the summer of 1971, Albany Medical College's Institute of Experimental Pathology and Toxicology oversaw the human safety evaluation of DOWCO 179, a Dow Chemical Company trade name for chlorpyrifos (Coulston et al., 1972). The study included 16 healthy adult male inmates from a pool of volunteers at Clinton Correctional Facility, a maximum-security prison in Dannemora, New York. The use of prisoners for an intentional human dosing study is unethical by current standards. However, this practice was common in the United States in the early 1970s, well before publication of the Belmont Report in 1979 and EPA's adoption of the Common Rule in 1991 (Oleskey et al., 2004).

The volunteers were randomized into four experimental groups: a control group (N = 4) assigned to receive a daily placebo, and three other groups (N = 4 for each) assigned to receive daily chlorpyrifos treatment at 0.014 mg/kg, 0.03 mg/kg, or 0.1 mg/kg body weight. Treatment periods started on different dates and lasted 49, 28, 22 and 10 days for the control, lowest, intermediate, and highest dose groups, respectively. Twice-weekly heparinized blood samples were analyzed by automated titration (Nabb and Whitfield, 1967) for two primary health endpoints: red blood cell cholinesterase (RBC-ChE) activity and plasma cholinesterase (plasma ChE) activity, both measured in units of μmoles acetate/min/ml. Following completion of the treatment phase, monitoring of ChE activity continued semiweekly until values returned to baseline levels. Statistical analysis of these data was conducted by Colin Park of Dow Chemical Company's Mathematical Applications Division (Park, 1972).

The study took place over 63 days (Table 1 and Fig. 1). Data from the control group were collected throughout the study period, but start and end dates differed among the three groups receiving chlorpyrifos. This design meant that, with the statistical tools available in 1972, it was not possible to compare the treatment groups with each other; rather, each treatment group could only be compared to concurrent controls.

Treatment effect was defined as a statistically significant ($p < 0.05$) depression of RBC-ChE or plasma ChE activity in treated subjects relative to controls by the F-test for the dose-by-day interaction term in a repeated measures analysis of variance (ANOVA). A repeated measures design means that successive measurements of ChE activity were noted for each subject over time, in this case at semiweekly (two-to-four-day) intervals. The ANOVA model analysis was computed laboriously by hand; it included random subjects with categorical day and dose variables. The F-test evaluated the general alternative of any kind of difference in the dose-by-day interaction, vs. the null hypothesis of a constant mean difference between treatment and control groups over time; that is, the F-test was not constrained to consider the more powerful and biologically plausible hypothesis that depression would increase over time in the treated group.

2.1. Coulston study findings

For RBC-ChE activity, treatment at the 0.1 mg/kg and 0.03 mg/kg doses produced no detectable change relative to controls. The researchers dismissed as an artifact the observation of significantly depressed RBC-ChE activity in the 0.014 mg/kg dose group, citing the imbalanced study design. They argued that the apparent difference for that group could be related to the extended time lag between baseline measurements (June 29 – July 7) and treatment measurements (July 29 – August 23) but was unlikely to be related to treatment, given that the two higher doses failed to show a significant effect.

Fig. 2 shows the Coulston study's measured trends in plasma ChE activity, the second health endpoint of interest. Each line represents a dose group's smoothed mean enzyme activity versus day of study. Plasma ChE activity declined rapidly in the highest treatment group (0.1 mg/kg), with two of the four subjects showing < 80% of baseline activity after the initial dosing. Treatment of the 0.1 mg/kg subjects was terminated after 9 days when plasma ChE levels averaged 34% of baseline. On Day 9 one of the four subjects reported symptoms consistent with ChE depression: runny nose, dizziness and faintness. The investigators attributed the symptoms to a cold; however, EPA scientists considered these symptoms to be treatment-related (USEPA 1984a).

Coulston et al. (1972) reported a statistically significant difference in plasma ChE activity between the control group and the 0.1 mg/kg dose group ($p < 0.05$). The report found no statistically significant difference between the control group and the 0.014 mg/kg dose group, and no statistically significant difference between the control group and the 0.03 mg/kg dose group. This latter finding was the basis for the authors' conclusion that 0.03 mg/kg was the NOAEL.

Given the importance of the 0.03 mg/kg treatment group to assignment of the NOAEL, it is of particular interest to examine the investigators' comparison between mean plasma ChE levels for that treatment group and the control group. They compared these levels on a day-by-day basis for all time points during treatment and computed a mean rate of depression for the entire treatment period; that is, equal weight was given to measurements at the beginning and the end of the 20-day treatment period. Using this approach the investigators reported that plasma ChE activity was reduced to 87% in the treatment group. However, on the final day of measurements, average plasma ChE activity for the treatment group was much lower; only 77% of the control average. Furthermore, as the authors note, by the end of the 21-day study period "plasma cholinesterase activity averaged 70% of the baseline levels." Despite this finding of substantial plasma cholinesterase inhibition, the authors conclude that there was "no significant toxicological effect" at the 0.03 mg/kg dose level. The levels of depression observed in this study would have triggered workplace investigations under California regulations during the 1970 s (CDPR, 2019).

2.2. Repeat of original analysis by repeated measures ANOVA

Our attempts to replicate the reported results by repeated measures ANOVA were successful in all groups only after recoding some dates and, in the 0.03 mg/kg group, omitting valid baseline data (See Table 1 for successful date coding). With data recoded to align all baseline measurements on two common baseline days, Park's results for the 0.1 mg/kg group are exactly replicable (see Table 2 for summarized results). In the 0.03 mg/kg group comparison, Park chose to exclude all data for the second baseline day from the analysis (Table 1); the result of this omission is that the difference in mean plasma ChE activity between the treatment and control groups over time is not statistically significant, with $p = 0.12$. If the second day's baseline data are included, however, the difference is statistically significant at $p = 0.012$. Results for the 0.014 mg/kg dose group were exactly replicable when we ignored control subject 16′s first baseline datum and recoded his second baseline day and first placebo day as baseline days 1 and 2, respectively.

The inconsistency in selection of data for the analysis – use of two baseline measurements for the 0.1 mg/kg and 0.014 mg/kg dose groups, but only one baseline measurement for the 0.03 mg/kg dose group – is unexplained, and frankly inexplicable. All of the pre-treatment values for the 0.03 mg/kg were valid measurements. Removal of 10% of the valid data (Table 2) reduced the power of the analysis,

Table 1
Plasma ChE measurements included in Park's three original comparisons by repeated measures ANOVA: 0.014 mg/kg/day subjects vs. controls, 0.03 mg/kg/day subjects vs. controls, and 0.1 mg/kg/day subjects vs. controls. NOTE: individual measurements in this table have been redacted to conform to requirements associated with receipt of this study through the Freedom of Information Act.

day of measurement		control subjects				0.014 mg/kg subjects				control subjects				0.030 mg/kg subjects				control subjects				0.100 mg/kg subjects			
date	day	2	4	9	16	7	8	14	15	2	4	9	16	10	11	12	13	2	4	9	16	1	3	5	6
29-Jun	0	X.X	X.X	X.X	X.X	X.X	X.X			X.X	X.X	X.X	X.X	X.X	X.X	X.X	X.X	X.X	X.X	X.X	X.X	X.X	X.X	X.X	X.X
1-Jul	2				X.X			X.X	X.X				X.X	X.X	X.X	X.X	X.X				X.X				
2-Jul	3	X.X	X.X	X.X		X.X	X.X			X.X	X.X	X.X						X.X	X.X	X.X		X.X	X.X	X.X	X.X
7-Jul	8	X.X	X.X	X.X	X.X			X.X	X.X	X.X	X.X	X.X	X.X	X.X	X.X	X.X	X.X	X.X	X.X	X.X	X.X				
9-Jul	10	X.X	X.X	X.X	X.X					X.X	X.X	X.X	X.X	X.X	X.X	X.X	X.X	X.X	X.X	X.X	X.X				
12-Jul	13	X.X	X.X	X.X	X.X					X.X	X.X	X.X	X.X	X.X	X.X	X.X	X.X	X.X	X.X	X.X	X.X				
14-Jul	15																					X.X	X.X	X.X	X.X
16-Jul	17	X.X	X.X	X.X	X.X					X.X	X.X	X.X	X.X	X.X	X.X	X.X	X.X	X.X	X.X	X.X	X.X	X.X	X.X	X.X	X.X
19-Jul	20	X.X	X.X	X.X	X.X					X.X	X.X	X.X	X.X	X.X	X.X	X.X	X.X	X.X	X.X	X.X	X.X	X.X	X.X	X.X	X.X
22-Jul	23	X.X	X.X	X.X	X.X					X.X	X.X	X.X	X.X	X.X	X.X	X.X	X.X	X.X	X.X	X.X	X.X	X.X	X.X	X.X	X.X
24-Jul	25	X.X	X.X	X.X	X.X					X.X	X.X	X.X	X.X	X.X	X.X	X.X	X.X	X.X	X.X	X.X	X.X	X.X	X.X	X.X	X.X
26-Jul	27	X.X	X.X	X.X	X.X					X.X	X.X	X.X	X.X	X.X	X.X	X.X	X.X	X.X	X.X	X.X	X.X	X.X	X.X	X.X	X.X
29-Jul	30	X.X	X.X	X.X	X.X	X.X	X.X	X.X	X.X	X.X	X.X	X.X	X.X	X.X	X.X	X.X	X.X	X.X	X.X	X.X	X.X	X.X	X.X	X.X	X.X
2-Aug	34	X.X	X.X	X.X	X.X	X.X	X.X	X.X	X.X	X.X	X.X	X.X	X.X	X.X	X.X	X.X	X.X	X.X	X.X	X.X	X.X	X.X	X.X	X.X	X.X
5-Aug	37	X.X	X.X	X.X	X.X	X.X	X.X	X.X	X.X	X.X	X.X	X.X	X.X	X.X	X.X	X.X	X.X	X.X	X.X	X.X	X.X	X.X	X.X	X.X	X.X
9-Aug	41	X.X	X.X	X.X	X.X	X.X	X.X	X.X	X.X	X.X	X.X	X.X	X.X	X.X	X.X	X.X	X.X	X.X	X.X	X.X	X.X	X.X	X.X	X.X	X.X
12-Aug	44	X.X	X.X	X.X	X.X	X.X	X.X	X.X	X.X	X.X	X.X	X.X	X.X					X.X	X.X	X.X	X.X	X.X	X.X	X.X	X.X
16-Aug	48	X.X	X.X	X.X	X.X	X.X	X.X	X.X	X.X	X.X	X.X	X.X	X.X	X.X	X.X	X.X	X.X	X.X	X.X	X.X	X.X	X.X	X.X	X.X	X.X
19-Aug	51	X.X	X.X	X.X	X.X	X.X	X.X	X.X	X.X	X.X	X.X	X.X	X.X	X.X	X.X	X.X	X.X	X.X	X.X	X.X	X.X	X.X	X.X	X.X	X.X
23-Aug	55	X.X	X.X	X.X	X.X	X.X	X.X	X.X	X.X	X.X	X.X	X.X	X.X	X.X	X.X	X.X	X.X	X.X	X.X	X.X	X.X	X.X	X.X	X.X	X.X
26-Aug	58	X.X	X.X	X.X	X.X	X.X	X.X	X.X	X.X	X.X	X.X	X.X	X.X	X.X	X.X	X.X	X.X	X.X	X.X	X.X	X.X	X.X	X.X	X.X	X.X
30-Aug	62	X.X	X.X	X.X	X.X	X.X	X.X	X.X	X.X	X.X	X.X	X.X	X.X					X.X	X.X	X.X	X.X				

first baseline measurement
second baseline measurement
baseline measurement omitted from 0.030 mg/kg group ANOVA
treatment period measurements
recovery period measurements (not included in analysis)

leading to the incorrect conclusion that there was no statistically significant difference between this treatment group and its concurrent controls.

2.3. Updated analysis by linear mixed effects regression

The linear mixed effects model (LMM) is a modern approach to conducting repeated measures ANOVA. Similar to the repeated measures ANOVA, it includes a random subject term to compensate for the lack of independence between repeated measurements on the same individual. Popularized by Laird and Ware in the early 1980's for its flexible formulation and efficient computational approach, the LMM estimates regression coefficients by maximum likelihood (Laird and Ware 1982, Fitzmaurice and Molenberghs, 2008). Because it tolerates unbalanced data, a LMM is better suited to the temporally misaligned Coulston study data than the traditional hand calculations used by Park. Using all of the study's baseline and treatment measurements in a single model, the LMM regression simultaneously estimates treatment effects for all three dose groups and controls. Furthermore, it is straightforward to parameterize LMMs to test the biologically plausible hypothesis that the treatment effect would be cumulative over time, a hypothesis that was difficult to evaluate with the tools available in 1972. Our LMM implementation tests this biologically plausible hypothesis by assuming that the dose accumulates linearly over time; i.e., plasma ChE activity decreases linearly as a function of dose and the number of days since first treatment.

Our LMM analyses employ two different indices of time: the categorical 'day of study' index (t in Table S1) in the term capturing day-to-day variability in activity readings — to account for laboratory effects; and a continuous count of treatment days (t_{trt} in Table S1) used in the dose*days expression of scientific interest. The day of study term permits adjustment for the confounding effect of time in this temporally misaligned study design without assuming any pattern in the portion of day-to-day measurement variation that is unrelated to treatment. We evaluate different approaches to estimating this laboratory effect adjustment given the limited number of daily measurements. As also discussed in the Supplementary Materials, the model approaches are 1) "pre-adjusted", where we subtracted laboratory effects estimated in controls alone from all observations, 2) "model-adjusted", where we used a standard regression adjustment for confounding, and 3)

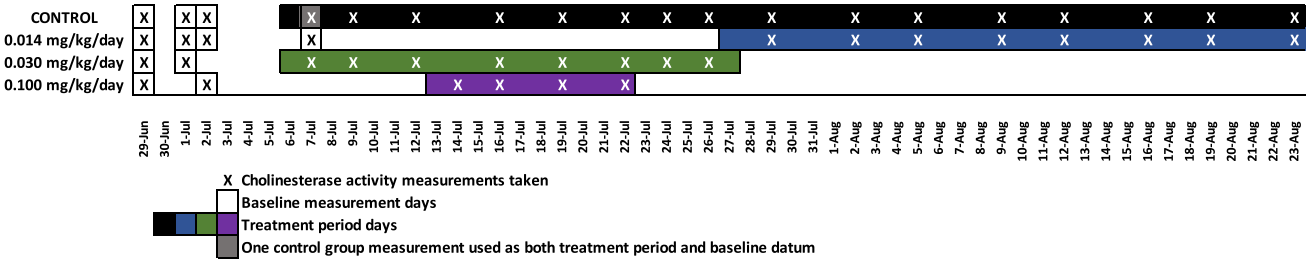

Fig. 1. Timing of treatment periods and ChE activity measurements by dose group.

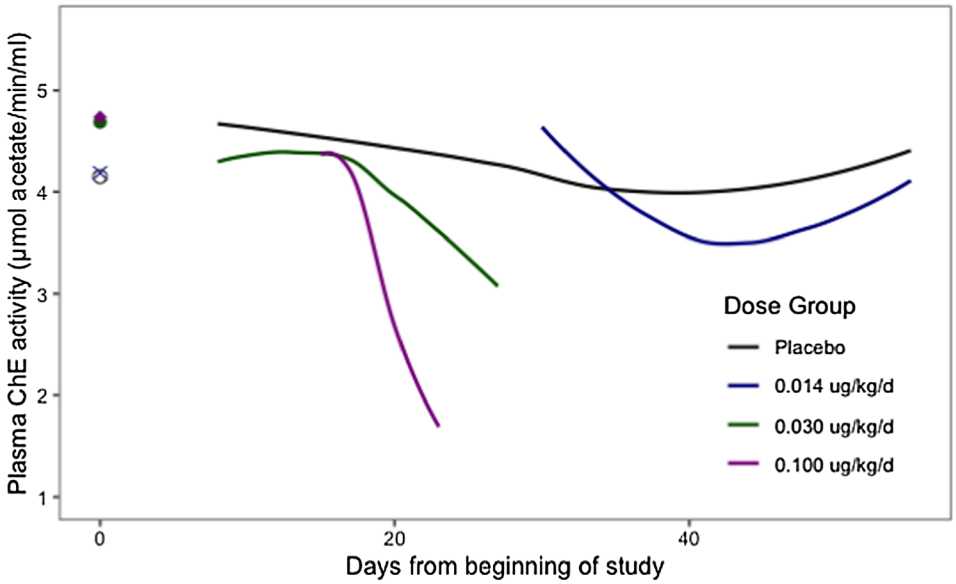

Fig. 2. Unadjusted mean plasma ChE measurements by dose group and day of study. Points are all baseline measurements and lines represent smoothed group means over group-specific treatment periods.

"unadjusted", which omitted any adjustment for the laboratory effects. The treatment day term is the scientifically important time index that allows assessment of the cumulative effect of chlorpyrifos treatment on plasma ChE activity. In distinct analyses we estimated the dose-related plasma ChE inhibition over time with dose considered as either a categorical or a continuous variable. The continuous dose model allowed us to evaluate whether the amount of plasma ChE inhibition over time was linear in dose. Further details of all these LMM analyses can be found in the Supplementary Materials.

Fig. 3 shows the adjusted smoothed means for all dose groups overlaid with trend lines estimated by LMMs fit to the pre-adjusted activity measurements. These two LMMs were fit to the activity measurements after they had been adjusted by the day-of-study effect estimated for the control group (pre-adjusted model). Fig. 3a shows the trend estimated by the categorical dose model. Fig. 3b shows the trend estimated by the continuous dose model, which assumes that the cumulative effect on ChE activity increases linearly with dose. Within each dose group the decline in ChE activity over the treatment period is approximately linear, and in both models the slopes are ordered such that the decline is steepest for the 0.1 mg/kg/day group and shallowest for the 0.014 mg/kg/day group. The two models give similar results, suggesting that it is reasonable to assume cumulative ChE activity increases linearly with dose (Table S2). For a daily dose of 0.1 mg/kg, the continuous dose model estimates a decline in plasma ChE of 0.294 umoles/min/ml/day (95% CI: −0.338, −0.249), while the categorical dose model estimates a decline of 0.331 umoles/min/ml/day (95% CI: −0.391, −0.270). For a daily dose of 0.03 mg/kg, the estimates are 0.086 (95% CI: –0.099, –0.073) and 0.110 (95% CI: −0.144, −0.077), respectively. Notably, both models also estimate statistically significant declines for the lowest daily dose of 0.014 mg/kg: the continuous model estimates a decline of 0.039 umoles/min/ml/day (95% CI: –0.046, –0.031), and it is 0.026 (95% CI: −0.043, −0.009) in the categorical dose model. Neither model suggests that the study has identified a NOAEL. Figure S1 shows generally similar results for our other approaches to handling the laboratory effects (adjusted and unadjusted) and Table S2 gives the slope estimates and 95% CIs for all six of the models we considered. We note that the small sample size (4 individuals per group) and the unbalanced design contribute to some variation in estimates across the six models.

3. Discussion

3.1. Coulston study reanalysis

We highlight several concerns with the Coulston Study. First, because treatment dates and durations varied among the dose groups, the original analysis was not able to estimate differences in effect between treatment groups; that is, only comparisons between each treatment group and the controls were possible. This restriction eliminated the study's potential to identify a graded association between dose and treatment effect.

Second, the necessity to omit unbalanced data from an already small data set yielded error estimates that were noisier than necessary, and therefore reduced the power to identify a treatment effect by repeated measures ANOVA. As discussed previously, the repeated measures ANOVA is already evaluating a less powerful alternative hypothesis because it is not constrained to focus on a biologically plausible treatment-related decrease in plasma ChE activity over time.

Third, the statistical analysts made the unexplained and highly consequential decision to omit eight valid baseline measurements from

Table 2
Results of repeated measures ANOVA examples for the dose by day interaction term (i.e. the treatment effect) for the original analysis and the replication of this analysis using all the available data for each dose-specific analysis.

Dose (mg/kg/day)	*Original Analysis*				*Replication of Original Analysis*			
	n	degrees of freedom*	F-value	p-value	n	degrees of freedom*	F-value	p-value
0.014	80	9	1.05	0.41	80	9	1.05	0.41
0.03	72	8	1.70	0.12	80	9	2.66	0.012
0.1	48	4	18.57	< 0.001	48	4	18.57	< 0.001

*Degrees of freedom for day x dose parameter = number of treatment days – 1.

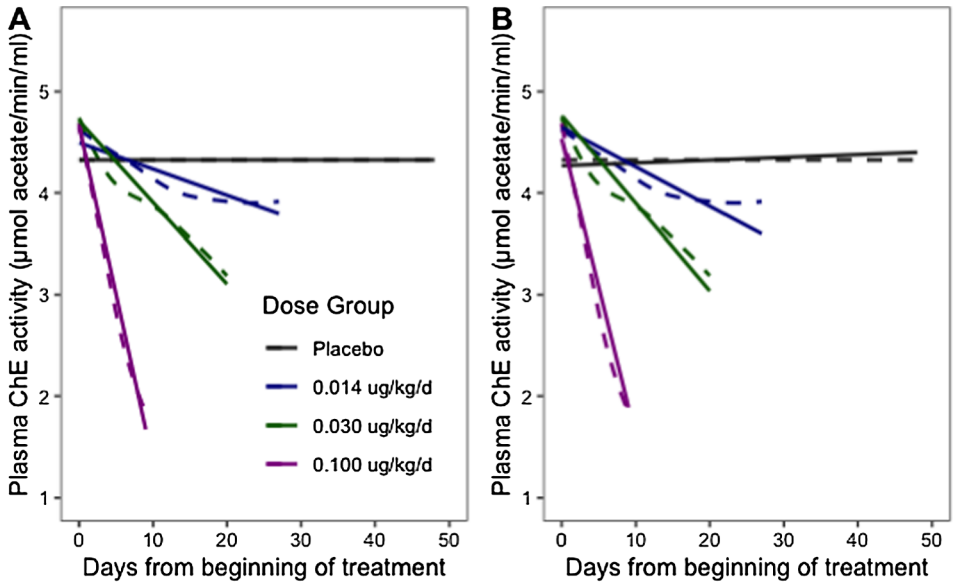

Fig. 3. Estimated treatment effects over time by dose group fit to the pre-adjusted data (solid lines) compared to smoothed means of these data (dashed lines) for A) categorical dose model, and B) continuous dose model.

the analysis for only one of the three treatment groups (0.03 mg/kg); this omission led to incorrect assignment of the NOAEL. Such an omission of valid data without justification is a form of data falsification that violates all standard codes of ethical research practice and is classified as outright research misconduct (NIH, 2020). Our findings, using both the original analysis approach and a technique more tolerant of unbalanced data, disagree with the original determination of no response to treatment at the 0.03 mg/kg/day dose, and our LMM analyses demonstrate a treatment effect at 0.014 mg/kg/day.

At a minimum, correct application of the original method to identify the treatment effect at 0.03 mg/kg/day would have reduced the NOAEL two-fold: from 0.03 mg/kg to 0.014 mg/kg. Our reanalysis using LMM suggests that the data could have justified even further lowering of the NOAEL: while the estimated trend in plasma ChE at 0.014 mg/kg/day varies somewhat across models and confidence intervals estimates are wider in the categorical dose models, five of the six slope estimates are significantly different from zero and the estimates provided by the linearly constrained dose models are unambiguously less than zero. That is, using a biologically plausible hypothesis that chlorpyrifos exposure accumulates over time, the Coulston Study did not identify a NOAEL for plasma cholinesterase inhibition.

3.2. Chlorpyrifos regulatory history

Table 3 provides a timeline for important events related to chlorpyrifos registration. Chlorpyrifos was first registered in 1965 under the Federal Insecticide, Fungicide and Rodenticide Act (FIFRA) by the U.S. Department of Agriculture for insect control in lawn, turf and selected crops. Authority for pesticide regulation was transferred to the U.S. Environmental Protection Agency in 1972. Until 1997 chlorpyrifos was registered for a variety of applications, including residential indoor and outdoor insect control and treatment for structural pests such as termites. The Food Quality Protection Act of 1996 brought increased attention to the multiple pathways by which children were exposed to chlorpyrifos. In 1997 EPA requested that the registrant withdraw several uses due to concerns over children's exposures in residential environments (USEPA, 1997a). Additional residential uses were suspended in 2000, and in 2002 new restrictions were imposed for chlorpyrifos use on certain crops (USEPA, 2006).

The Coulston Study (Coulston et al., 1972) has a curious history in the pesticide regulatory process, beginning with its almost immediate use in an early effort to estimate chlorpyrifos health risks. In 1972 the Food and Agriculture Organization of the World Health Organization cited the report in a review of pesticides in food (FAO/WHO, 1973). The review concluded that 0.03 mg/kg-day was the "minimal threshold response level in humans," and that the lowest dose in the study – 0.014 mg/kg-day – was the NOAEL. However, there is no indication that the study design or statistical procedures were scrutinized during this review.

Although never published or peer-reviewed, the Coulston Study has appeared frequently in the reference section of peer-reviewed journal articles. Each of these articles treats the Coulston Study as if it were a peer-reviewed study, accepting its conclusions without conducting a critical analysis of the study's design or statistical procedures (Vaccaro, 1993; Clegg and van Gemert, 1999a,b; van Gemert et al., 2001; Zhao et al., 2005,2006; Eaton et al., 2008). In one memorandum the EPA itself mistakenly classified the Coulston Study as a peer-reviewed publication (USEPA, 1998b). The EPA bibliography lists 18 unpublished reports by their master record identifier (MRID) numbers. It then has a separate section called "Published Literature Studies". The Coulston Study is one of two documents listed in this section, despite its also having a MRID number. The other study cited in the section is a peer-reviewed journal article.

3.3. US EPA evaluation of the Coulston study

The U.S. Environmental Protection Agency adopted the Coulston Study as the basis for human health chlorpyrifos risk assessments between 1984 and 1999 (USEPA, 1999a). There is no record that the study design or statistical procedures employed were scrutinized critically during that time by the EPA.

In May 1984 the Toxicology Branch at the U.S. EPA reviewed the Coulston Study and concluded that 0.03 mg/kg-day was the "apparent NOAEL" (USEPA, 1984a). However, the EPA reviewer considered the depression of plasma ChE at the 0.03 mg/kg dose to be "equivocal" because of the small sample size and high variability in ChE measurements. He therefore recommended that the study be classified as "Supplementary Data". Surprisingly, less than a week later, the same reviewer proposed that the Coulston Study chronic NOAEL of 0.03 mg/kg-day be used as part of the Chlorpyrifos Registration Standard, and the proposal omitted any mention of study weaknesses (USEPA, 1984b). Thus, the Coulston Study's NOAEL was established as the point

Table 3
Timeline for chlorpyrifos registration and use in the United States.

1965	Registration by USDA Dow Chemical Company for lawn/turf grasses/selected crops	USEPA 2006
1970	U.S. EPA formed; transfer of pesticide registration from USDA to EPA	USEPA 1985
1972	Completion of DOWCO 179 safety evaluation study in humans (Coulston Study)	Coulston et al. 1972
1972	New requirement that registrants test pesticides to demonstrate "no unreasonable adverse effects on human health" when used as directed	USEPA 1985
1973	World Health Organization uses Coulston study to establish a NOAEL of 0.014 mg/kg	FAO/WHO 1973
1975	Registration as flea treatment for pets	NPIC 2019
1982	Laird and Ware describe a maximum likelihood algorithm for computingLMM estimates	Laird and Ware 1982
1986	EPA uses the NOAEL of 0.03 mg/kg from the Coulston report for its point of departure in risk assessments	USEPA, 1998a
1987	EPA allows multiple uses of chlorpyrifos in residential environments	USEPA 1987
1987	CA Department of Food and Agriculture and Department of Health raise concerns about indoor uses of OP pesticides	Knaak et al. 1987
1988	Chlorpyrifos becomes the primary chemical for structural control when all registered uses of chlordane are cancelled	ATSDR 2019
1996	Food Quality Protection Act requires reassessment of all existing pesticide tolerances and additional safety factor for children	USEPA, 1998a
1997	Many indoor residential uses withdrawn by registrant in light of new risk assessment methods required by FQPA	USEPA 1997a
1998	Reference dose reduced to 0.0003 mg/kg-day to comply with FQPA requirement to protect children	USEPA, 1998a
2000	Revised human health risk assessment based on animal data results in withdrawal of most residential use registrations	USEPA 2006
2002	EPA issues new restrictions for chlorpyrifos use on certain crops	USEPA 2006
2006	EPA establishes Human Studies Review Board (HSRB) to evaluate all studies that involve intentional human exposure or dosing	USEPA 2019
2009	Dow withdraws Coulston study from consideration by HSRB	USEPA 2009
2016	EPA Health Hazard Evaluation: scientists recommend new RfD that would result in discontinuation of all uses of chlorpyrifos	USEPA 2016
2017	EPA administrator maintains existing registrations despite advice from EPA scientists	USEPA 2017
2018	Courts rule that EPA must use existing science to justify regulatory decision	Lulac v Wheeler 2018

of departure for all non-acute chlorpyrifos risk assessments conducted by the Agency.

The consequences of this decision were soon evident with EPA's continued registration of chlorpyrifos for multiple residential uses, including termite control, pet spray and dip, indoor crack-and-crevice applications, total release foggers, and indoor broadcast applications (USEPA, 1987).

As late as February 1998, EPA's Hazard Assessment Review Committee stated, "The [chlorpyrifos] RfD [reference dose] was derived from a NOAEL of 0.03 mg/kg/day based on plasma cholinesterase inhibition observed in human volunteers" (USEPA, 1998a). A subsequent statement by this committee in December of that year reaffirmed the use of the Coulston Study as a point of departure for chlorpyrifos risk assessments (USEPA, 1998b).

3.4. Residential use of chlorpyrifos as a public health concern

Although chlorpyrifos passed through the regulatory process without much debate, there was growing evidence that it might pose a health hazard in residential environments. A 1986 memo from the EPA Toxicology Branch (USEPA 1986) titled, "Human Health Risks for Chlorpyrifos" stated, "The PIMS [Pesticide Incident Monitoring System] tabulation indicated that of the 421 incidents reported between 1966 and 1981, 129 were the result of exposure to chlorpyrifos alone." This represented 31% of the reports. The Agency's memo also noted that chlorpyrifos "caused a total of 34 days of hospitalization between the years 1980 and 1985." The memo then proceeded to list a number of these reports. Of particular interest are three reports involving infants and small children.

> During application of chlorpyrifos (2 oz/gal) to carpeting by a PCO [Pest Control Operator], a 1–1/2 year old infant was accidentally dermally exposed. The infant died the next day. At autopsy, findings of pulmonary edema were reported, and a diagnosis of organophosphate intoxication was made.
>
> A family brought a new-born infant home to their residence which had been treated three days previously by a 'pest control firm'. The infant became progressively more ill over the next several days and was admitted to the hospital in apparently critical condition. His symptoms included cyanosis, respiratory arrest, limpness, unresponsiveness, and pinpoint pupils. The infants [sic] difficulties were complicated by an apparent mis-diagnosis of his condition, however he reportedly responded "promptly" to treatment with atropine, the standard treatment for organophosphate intoxication.
>
> A residence was treated by 'exterminators' with a mixture of chlorpyrifos and dichlorvos. The bedroom of a 3-month-old child was not treated, and the infant was removed from the residence for seven hours after treatment. Over the next 4 days, the infant developed symptoms of runny nose, diarrhea, weakness and lethargy. The child was admitted to the hospital in apparently poor condition, with symptoms of respiratory depression, stupor, excess secretions, pneumonia and bradycardia. The infant reportedly responded well to treatment with atropine and pralidoxime, standard treatments for organophosphate intoxication.

Why, in light of such information, did the Agency fail to conduct a more thorough assessment of chlorpyrifos toxicity and its residential uses? Apparently, the Agency felt that it lacked critical information for its risk assessments. The 1987 EPA Guidance Document explained:

> Regarding the concerns for total human exposure, the Agency has decided that it is not appropriate at this time to initiate a Special Review for chlorpyrifos because of the lack of sufficient residue and exposure data that are needed to assess the potential hazards to humans.

In short, the lack of adequate exposure data deterred the Agency from making an in-depth analysis of chlorpyrifos. Had a Special Review been initiated, perhaps the flaws in the Coulston Study would have been discovered. Instead, the Agency allowed multiple residential uses of chlorpyrifos, despite acknowledging that insufficient data were available to assess the potential hazards to humans.

Even in the absence of satisfactory exposure data, concern continued to grow regarding health risks from residential pesticide use. A 1989 case study described pesticide intoxication in a residential environment (Wagner and Gallant, 1989). The authors noted that "the pesticide was used according to the label instructions. It was, however, used repeatedly in a closed area. The medical community may benefit from the knowledge that seemingly appropriate use of a readily available household spray in the residence … may result in toxic exposure". A 1990 review of data from the National Pesticide Hazard Assessment Program found that most reported cases of pesticide-related illness occurred in the home (Wagner 1990). Chlorpyrifos was responsible for more cases than any of the other 38 insecticides listed, with the exception of chlordane, a chemical that had been used for decades as a structural pest control agent, and whose EPA registrations were cancelled in 1988 following its identification as a potential carcinogen (ATSDR 2019).

3.5. California concerns regarding indoor residential pesticide use

In the mid-1980s, the California Department of Food and Agriculture became very concerned with the use of organophosphorus (OP) and carbamate pesticides inside residences (Knaak et al. 1987). They called representatives from Dow Chemical Company and Mobay Chemical Company (now Bayer), among others, to discuss the registration of chlorpyrifos (Dow) and propoxur (Mobay), particularly for broadcast spraying inside homes. Scientists at Mobay conducted a risk assessment for propoxur, concluded that risks were too high for infants and young children, and subsequently withdrew its registration request for broadcast use (Berteau et al. 1989).

Chlorpyrifos, however, remained registered for broadcast application and all other residential uses. In 1997 EPA reported that "Dursban sales have increased 26 fold since 1975" (USEPA 1997b). The same review affirmed that "chlorpyrifos is one of the leading causes of acute insecticide poisoning incidents in the United States." It went on to state that 1966 unintentional exposures to chlorpyrifos for children less than six years of age were reported by Poison Control Centers in 1993, and that this number increased to 2348 in 1994. Furthermore, in 1995, EPA fined Dow $732,000 for failure to report numerous additional incidents of pesticide-related illness (USEPA, 1995). The weight of evidence by this time seemed to indicate that EPA's regulation of chlorpyrifos failed to adequately protect children's health. Only in 1997, however, did EPA cancel the use of chlorpyrifos for broadcast applications (USEPA, 1997a). We will never know the number of illnesses that could have been avoided had such uses been discontinued in the late 1980s as they were for propoxur.

3.6. Current chlorpyrifos risk assessments

Current EPA risk assessments of chlorpyrifos continue to view cholinesterase inhibition (both plasma and red blood cell) as the most sensitive endpoint. EPA's Registration Eligibility Decision cites multiple rodent and dog studies submitted by the registrant as the basis for the chlorpyrifos chronic NOAEL (USEPA, 2006). Mie and Rudén (2018) recently criticized the quality of one registrant-sponsored study, suggesting that such studies have not been reviewed thoroughly. Their analysis, along with our analysis of the Coulston Study, indicate the need for independent review of all registrant-sponsored studies used in the setting of standards.

Numerous animal toxicity studies suggest endpoints for chlorpyrifos that are more sensitive than cholinesterase inhibition, a topic that is beyond the scope of this paper. Furthermore, recent credible epidemiologic evidence has associated adverse neurodevelopmental outcomes with prenatal exposures to chlorpyrifos and other OP pesticides (2011, 2015; Rauh et al., 2006; Rauh, 2018; Hertz-Picciotto et al., 2018). As mentioned in our Introduction, EPA scientists did propose a new chronic health endpoint for chlorpyrifos based on epidemiologic studies (USEPA, 2016), but this analysis has not yet been used as a basis for chlorpyrifos risk assessments.

3.7. Key findings

At the outset of this paper we posed three questions: Why did a study so critical to risk assessment and protection of the public health never receive a rigorous review? What were the consequences of this failure to review? Are there safeguards in place to assure that evidence used in risk assessments is produced and reported in accordance with accepted scientific procedures?

The review procedures in place at EPA during the time period when the Coulston Study was used for risk assessments were entirely internal. It appears that the report was not reviewed by a statistician, as there are no comments in the EPA documents regarding study design or statistical procedures. An independent scientific evaluation of the Coulston Study might well have identified the weaknesses in the study design and the omission of valid baseline data for the key treatment group.

We can only speculate on the impact that a lower NOAEL would have had on EPA's decision to register chlorpyrifos for multiple residential uses. It does seem likely that a lower point of departure would have obligated EPA to regulate more cautiously, and that chlorpyrifos exposures to small children, such as those documented by the Poison Control Center network and California regulators, could have been reduced.

The establishment of EPA's Human Subjects Rule in 2005 represented a major step forward in ensuring that human toxicology studies are reviewed thoroughly from both a scientific and ethical perspective (USEPA, 2019). The membership of the Human Studies Review Board (HSRB), formed as a part of this rule, consists of independent scientists and ethicists drawn from outside the Agency. The HSRB has access to the entire body of information underlying studies that are being considered as the basis for human health risk assessments. The Coulston Study has never been evaluated by the HSRB, as the registrant asked that it be removed from the list of studies to be reviewed (USEPA, 2009).

Many of the intentional human dosing studies submitted to EPA by registrants were equivalent to clinical trials overseen by the U.S. Food and Drug Administration (FDA). FDA requires that all proposed clinical trials be reviewed prior to initiation (2019). It is not clear that this same requirement is in place for human testing of pesticides. While such pesticide toxicity studies require approval of an Institutional Review Board, there does not appear to be a requirement that the study design be submitted to EPA or its Human Studies Review Board for review and approval prior to initiation (USEPA, 2020). Such a requirement would greatly strengthen the integrity of such registrant-sponsored studies.

4. Conclusions

Our reanalysis of the intentional human dosing study documented in the Coulston Study demonstrates that the NOAEL for plasma cholinesterase inhibition is likely to be lower than 0.014 mg/kg, rather than the 0.03 mg/kg used between 1984 and 1999 for EPA risk assessments. The Coulston Study misled regulators by omitting valid data for the key treatment group (0.03 mg/kg), resulting in a finding of no effect. Our updated analysis indicates that even the lowest dose was unlikely to be a NOAEL. A proper analysis of the Coulston Study would have lowered or eliminated the NOAEL. Either action would have reduced the acceptable dose for chlorpyrifos, and may well have led to more restrictions on its use, particularly in scenarios where infants and children were exposed, including indoor broadcast and crack-and-crevice spraying, pet treatment, and structural application for termite control. An earlier reduction in the NOAEL and increased exposure mitigation would have likely reduced the incidence of adverse health effects in children of that era. It is tragic that an omission of valid data from the analysis of the Coulston Study may have adversely impacted public health for at least 15 years.

CRediT authorship contribution statement

Lianne Sheppard: Conceptualization, Methodology, Resources, Software, Formal analysis, Supervision, Visualization, Writing - review & editing. **Seth McGrew:** Data curation, Software, Investigation, Formal analysis, Visualization, Writing - original draft, Writing - review & editing. **Richard A. Fenske:** Conceptualization, Resources, Writing - original draft, Writing - review & editing.

Declaration of Competing Interest

The authors declared that there is no conflict of interest.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envint.2020.105905.

References

ATSDR 2019. Toxic Substances Portal – Chlordane. Agency for Toxic Substances and Disease Registry, Centers for Disease Control and Prevention. Accessed on September 11, 2019: https://www.atsdr.cdc.gov/phs/phs.asp?id=353&tid=62.

Berteau PE, Knaak JB, Mengle DC. 1989. Insecticide absorption from indoor surfaces: hazard assessment and regulatory requirements. In: Wang RGM, Franklin CA, Honeycutt RC, Reinert JC (eds): Biological Monitoring for Pesticide Exposure. ACS Symposium Series 382. Washington DC: American Chemical Society.

CDPR. 2019. Medical Supervision. California Department of Pesticide Regulation. California Code of Regulations, Title 3, Division 6, Chapter 3, Subchapter 3, Article 2, Section 6728. Accessed September 11, 2019: https://www.cdpr.ca.gov/docs/legbills/calcode/030302.htm.

Clegg, D.J., van Gemert, M., 1999a. Determination of the reference dose for chlorpyrifos: proceedings of an expert panel. J. Toxicol. Environmental Health, Part B 2, 211–255.

Clegg, D.J., van Gemert, M., 1999b. Expert panel report of human studies on chlorpyrifos and/or other organophosphate exposures. J. Toxicology Environmental Health, Part B 2, 257–279.

CFR. 2019. Final Rule for Clinical Trials Registration and Results Information Submission. 42 Federal Code of Regulations Part 11. Accessed April 6, 2020. https://www.federalregister.gov/documents/2016/09/21/2016-22129/clinical-trials-registration-and-results-information-submission.

Coulston F, Golberg L, Griffin T. 1972. Safety evaluation of Dowco 179 in human volunteers. Unpublished report, the Institute of Experimental Pathology and Toxicology, Albany Medical College, Albany, NY. [Report obtained from U.S. EPA through Freedom of Information Act request].

Eaton, D.L., Danoff, R.B., Autrup, H., Bridges, J., Buffler, P., Costa, L.G., et al., 2008. Review of the toxicology of chlorpyrifos with an emphasis on human exposure and neurodevelopment. Crit. Rev. Toxicol. S2, 1–125.

FAO/WHO. 1973. 1972 Evaluations on some pesticide residues in food. Monographs. World Health Organization. Report of the 1972 Joint Meeting of the FAO Working Party on Pesticide Residues and the WHO Expert Committee on Pesticide Residues, WHO Technical Report No. 525; FAO Agricultural Studies, No. 90. Accessed January 19, 2019. http://www.inchem.org/documents/jmpr/jmpmono/v072pr10.htm.

Fitzmaurice, G., Molenberghs, G. (2008). Advances in longitudinal data analysis: an historical perspective. In Fitzmaurice, G., Davidian, M., Verbeke, G., & Molenberghs, G., Longitudinal data analysis (pp. 17-42). Chapman and Hall/CRC.

Hertz-Picciotto, I., Sass, J.B., Engel, S., Bennett, D.H., Bradman, A., Eskenazi, B., et al., 2018. Organophosphate exposures during pregnancy and child neurodevelopment: Recommendations for essential policy reforms. PLoS Med. 15 (10), e1002671. https://doi.org/10.1371/journal. pmed.1002671.

Knaak, J.B., Schreider, J., Berteau, P., 1987. Hazard assessment of indoor use of chlorpyrifos, dichlorvos, propoxur and other organophosphates and Nmethyl carbamates. Worker Health and Safety Branch Report No. HS-1423. California Department of Food and Agriculture, Sacramento, CA.

Laird, N.M., Ware, J.H., 1982. Random-effects models for longitudinal data. Biometrics 963–974.

LULAC v Wheeler. 2018. On Petition for Review of an Order of the Environmental Protection Agency. United States Court of Appeals for the Ninth Circuit. Argued and Submitted July 9, 2018, Seattle, Washington. Filed August 9.

Mie A, Rudén C, Grandjean P. 2018. Safety of Safety Evaluation of Pesticides: developmental neurotoxicity of chlorpyrifos and chlorpyrifos-methyl. Environ Health. 17(1):77. Published 2018 Nov 16. doi:10.1186/s12940-018-0421-y.

Nabb DP, Whitfield F. 1967. Determination of cholinesterase by an automated pH stat method. Archives of Environmental Health: An International Journal, 15(2):147-154.

NIH (2020, April 6). Research Misconduct – Overview. Retrieved from https://grants.nih.gov/policy/research_integrity/overview.htm.

NPIC. 2019. National Pesticide Information Center Product Research Online. Retrieved August 8, 2019 from http://npic.orst.edu/NPRO/.

Oleskey, C., Fleischman, A., Goldman, L., Hirschhorn, K., Landrigan, P.J., Lappe, M., Marshall, M.F., Needleman, H., Rhodes, R., McCally, M., 2004. Pesticide testing in humans: ethics and public policy. Environ. Health Perspect. 112 (8), 914–919.

PANNA v EPA, 2015. Pesticide Action Network North America v United States Environmental Protection Agency. Writ of Mandamus, US Court of Appeals for the Ninth Circuit, August 10.

PANNA/NRDC v USEPA. 2017. (Pesticide Action Network North America & Natural Resources Defense Council v U.S. Environmental Protection Agency) Petiitioners' Motion for Further Mandamus Relief. US Court of Appeals for the Ninth Circuit, April 5.

Park C. 1972. Analysis of Plasma and Red Blood Cell - Cholinesterase Levels in Humans Ingesting DOWCO 179. Unpublished report, The Dow Chemical Company, Mathematical Applications Division, Computation Research Laboratory, Midland, MI. [Report obtained from U.S. EPA through Freedom of Information Act request].

Rauh, V.A., Garfinkel, R., Perera, F.P., Andrews, H.F., Hoepner, L., Barr, D.B., 2006. Impact of prenatal chlorpyrifos exposure on neurodevelopment in the first 3 years of life among inner-city children. Pediatrics 118, e1845–e1859.

Rauh, V., Arunajadai, S., Horton, M., Perera, F., Hoepner, L., Barr, D.B., et al., 2011. Seven-year neurodevelopmental scores and prenatal exposure to chlorpyrifos, a common agricultural pesticide.". Environ. Health Perspect. 119 (8), 1196.

Rauh VA, Garcia WE, Whyatt RM, Horton MK, Barr DB, Louis ED. 2015. Prenatal exposure to the organophosphate pesticide chlorpyrifos and childhood tremor. Neurotoxicology. 51:80-6.

Rauh VA. 2018. Polluting Developing Brains — EPA Failure on Chlorpyrifos. N Engl J Med 378;13, March 29.

Roberts, J.R., Reigart, J.R., 2013. Recognition and Management of Pesticide Poisonings, Sixth ed. Environmental Protection Agency, Washington DC, Office of Pesticide Programs, U.S.

USEPA. 1984a. Review of Human Cholinesterase Study. Memorandum from GJ Burin, Toxicologist to WJ Burnam, Chief, Toxicology Branch, Health Effects Division, Office of Pesticides and Toxic Substances, May 18.

USEPA. 1984b. Chlorpyrifos Registration Standard. Memorandum from GJ Burin, Toxicologist, thru WL Burnam, Chief, Toxicology Branch, Health Effects Division, to J Ellenberger, Insecticide-Rodenticide Branch Registration Division, Office of Pesticides and Toxic Substances, May 24.

USEPA. 1985. EPA History (1970-1985). Available at https://archive.epa.gov/epa/aboutepa/epa-history-1970-1985.html. [Accessed August 12, 2019].

USEPA. 1986. Human Health Risks of Chlorpyrifos. Memorandum from S Saunders, Toxicology Branch to M McDavit, Special Review Branch, Registration Division, Office of Pesticides and Toxic Substances, April 10.

USEPA, 1987. Guidance for the Reregistration of pesticide products containing chlorpyrifos as the Active Ingredient 059101. Environmental Protection Agency Office of Pesticide Programs, Office of Pesticides and Toxic Substances.

USEPA. 1995. EPA fines DowElanco for failure to report pesticide health effects. Environmental News. Communications, Education and Public Affairs, U.S. Environmental Protection Agency, May 1.

USEPA. 1997a. Agreement reached between EPA and chlorpyrifos pesticide registrants. Press Advisory, Communications, Education and Public Affairs. U.S. Environmental Protection Agency, June 6.

USEPA 1997b. Review of chlorpyrifos poisoning data. Memorandum from J. Blondell and VA Dobozy to L Propst, Section Head, Reregistration Branch, Special Review and Reregistration Division. Office of Pesticide Programs, U.S. Environmental Protection Agency, January 14.

USEPA, 1998a. Chlorpyrifos - FQPA Requirement - Report of the Hazard Identification Assessment Review Committee. Office of Pesticide Programs. U.S., Environmental Protection Agency, Washington DC, February, pp. 2.

US EPA. 1998b. Chlorpyrifos - Re-evaluation - Report of the Hazard Identification Assessment Review Committee. Office of Pesticide Programs. (U.S. Environmental Protection Agency, Washington DC, December 7.

USEPA. 1999a. Chlorpyrifos – replacement of human study used in risk assessments – report of the Hazard Identification Review Committee. Memorandum from J Rowland and P Wagner to S Knizner, Branch Senior Scientist, Health Effects Division. Office of Pesticide Programs, U.S. Environmental Protection Agency, June 2.

USEPA. 2006.Finalization of Interim Reregistration Eligibility Decisions (IREDs) and Interim Tolerance Reassessment and Risk Management Decisions (TREDs) for the Organophosphate Pesticides, and Completion of the Tolerance Reassessment and Reregistration Eligibility Process for the Organophosphate Pesticides. Memorandum from D Edwards, Special Review and Reregistration Division, to J Jones, Office of Pesticide Programs,, July 31.

USEPA. 2009. Withdrawal of Coulston et al. Chlorpyrifos Toxicity Study from HSRB Agenda. Memorandum from D Edwards, Director, Office of Pesticide Programs, to PI Lewis, Designated Federal Officer, Human Studies Review Board, Office of the Science Advisor, Office of Pesticides and Toxic Substances, June 18.

USEPA. 2016. Chlorpyrifos: Revised Human Health Risk Assessment for Registration Review. Memorandum from W Britton et al. Health Effects Division, and C Tan, National Exposure Research Laboratory, Office of Research and Development, to D Friedman, Chemical Review Manager, Risk Management and Implementation Branch II, Pesticide Re-evaluation Division, Office of Chemical Safety and Pollution Prevention, November 3.

USEPA. 2017. Chlorpyrifos: order denying PANNA and NRDC's petition to revoke tolerances, 82 Fed. Reg. 16, 581 (April 5). Retrieved November 30, 2017. https://www.epa.gov/sites/production/files/2017-03/documents/chlorpyrifos3b_order_denying_panna_and_nrdc27s_petitition_to_revoke_tolerances.pdf.

USEPA. 2019. Basic Information about Human Subjects Research. Office of the Science Advisor. U.S. Environmental Protection Agency. Available at https://www.epa.gov/osa/basic-information-about-human-subjects-research-0 [Accessed July 9, 2019].

USEPA. 2020. Protection for Subjects in Human Research Involving Pesticides. U.S. Environmental Protection Agency. Accessed April 8, 2020. https://www.epa.gov/pesticide-advisory-committees-and-regulatory-partners/protections-subjects-human-research-pesticides.

Vaccaro JR. 1993. Risks associated with exposure to chlorpyrifos and chlorpyrifos formulation components. Chapter 25 in Pesticides in Urban Environments, Racke KD and Leslie AR, editors. ACS Symposium Series 522:297-306, American Chemical Society, Washington DC.

van Gemert, M., Dourson, M., Moretto, A., Watson, M., 2001. Use of human data for the derivation of a reference dose for chlorpyrifos. Reg. Toxicol. Pharmacol. 33, 110–116.

Wagner, S.L., Gallant, J.D., 1989. Organophosphate intoxication from over-the-counter insecticides. Ann. Emerg. Med. 18 (7), 802.

Wagner, S.L., 1990. Pesticide illness surveillance: review of the national pesticide hazard assessment program. Am. J. Ind. Med. 18 (3), 307–312.

Zhao, Q., Gadafui, B., Dourson, M., 2005. Lower birth weight as a critical effect of chlorpyrifos: a comparison of human and animal data. Regul. Toxicol. Pharm. 42, 55–63.

Zhao, Q., Dourson, M., Gadaghbi, B., 2006. A review of the reference dose for chlorpyrifos. Reg Toxicol Pharmacol 44, 111–124.