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COMMENTARY Open Access

Safety of Safety Evaluation of Pesticides: developmental neurotoxicity of chlorpyrifos and chlorpyrifos-methyl

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Original article https://www.ncbi.nlm.nih.gov/pubmed/30442131
Letter to the editor, Juberg et al. https://www.ncbi.nlm.nih.gov/pubmed/30871546
Response to Juberg et al. https://www.ncbi.nlm.nih.gov/pubmed/30944002

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Slides prepared by Axel Mie for HEAL webinar on 12th of June, 2019

Chlorpyrifos – toxicity. Acceptable Daily Intake (ADI)

EU:

ADI 2014: 0,001 mg/kg bw/day.

Critical effect: RBC AChE inhibition, 2 year rat/dog study, NOAEL 0,1 mg/kg bw/day

USA:

ssPAD 2016 (suggested): 0,0000012-0,000002 mg/kg bw/day (children/females 13-49 yrs).

Weight of evidence analysis. ssPAD based on IQ loss in children due to prenatal exposure, epidemiological study, PBPK, LOAEL/100

DNT guideline study 1998 (rats):

DNT NOAEL 5 mg/kg bw/day

Chlorpyrifos – DNT guideline study (Maurissen 2000) [1]

TOXICOLOGICAL SCIENCES 57, 250–263 (2000)

Lack of Selective Developmental Neurotoxicity in Rat Pups from Dams Treated by Gavage with Chlorpyrifos

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Received October 30, 1998; accepted June 28, 2000

Endpoints: General toxicity, developmental landmarks, behavioural tests in offspring, brain morphometrics (PND 11 + 65)

[1] Maurissen 2000 https://www.ncbi.nlm.nih.gov/pubmed/11006355

Chlorpyrifos – Maurissen 2000

TABLE 5
PND 11 Brain Measurements

Dosage (mg/kg/day)	Brain wt (g)	Ant./Post. cerebrum (mm)	Ant./Post. cerebellum (mm)	Frontal cortex (μm)	Parietal cortex (μm)	Caudate-putamen (μm)	Corpus callosum (μm)	Hippocampus (μm)	Cerebellum ht (μm)	Ext'l germ layer (μm)
Males										
0	1.28 ± 0.04	12.5 ± 0.3	3.3 ± 0.3	1348 ± 53	1336 ± 54	2240 ± 84	293 ± 25	904 ± 93	3504 ± 129	37 ± 2*
0.3	1.41 ± 0.07	13.4 ± 0.5	3.4 ± 0.4	1360 ± 100	1448 ± 58	2240 ± 108	303 ± 24	1004 ± 114	3456 ± 172	38 ± 4
1	1.36 ± 0.08	13.1 ± 0.5	3.3 ± 0.2	1352 ± 47	1448 ± 33	2312 ± 93	290 ± 36*	972 ± 54	3416 ± 200	40 ± 7
5	1.17 ± 0.16[a,]*	11.8 ± 1.0*	2.5 ± 0.6[a,]*	1272 ± 153*	1256 ± 138*	2224 ± 147*	293 ± 56	824 ± 66*	3008 ± 504[a,]*	38 ± 3
Females										
0	1.28 ± 0.08	12.4 ± 0.3	3.2 ± 0.2	1376 ± 92	1380 ± 54	2384 ± 131	307 ± 38	936 ± 82	3512 ± 200	39 ± 3
0.3	1.28 ± 0.04	12.7 ± 0.3	3.0 ± 0.3*	1388 ± 79	1376 ± 20	2224 ± 116	286 ± 27	912 ± 50	3176 ± 130	36 ± 6*
1	1.27 ± 0.11	12.8 ± 0.7	3.3 ± 0.2	1356 ± 54*	1368 ± 80	2288 ± 108	304 ± 36	932 ± 96	3120 ± 328[a,]*	41 ± 6
5	1.17 ± 0.13[a,]*	12.2 ± 0.6*	3.0 ± 0.3*	1368 ± 86	1304 ± 72*	2152 ± 134[a,]*	274 ± 40*	828 ± 79*	3208 ± 226	41 ± 6

Notes. Values given are mean ± SD. Overall statistically significant ANOVA for males (Brain wt, Ant./Post. cerebrum, Ant./Post. cerebellum, Parietal cortex, Hippocampus, and Cerebellum ht) and females (Brain wt, Caudate-putamen, and Cerebellum ht) separately ($\alpha = 0.05$).

[a]Statistically significant Dunnett's test ($\alpha = 0.05$).

*Smallest values.

DNT study report requested from Swedish Chemicals

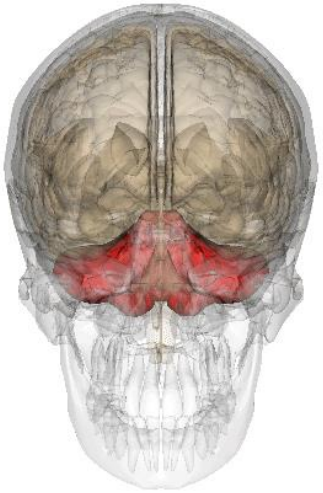
Agency under freedom of information legislation.

Human cerebellum

Functions:

Motor control: coordination, precision, accurate timing

Also involved in various cognitive processes



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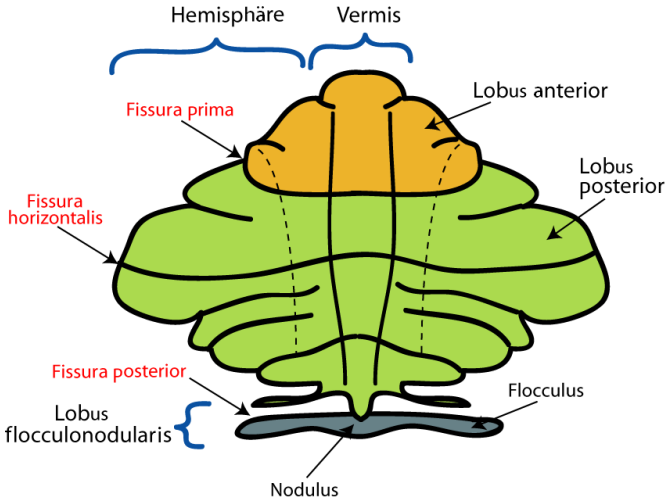

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Cerebellum height – no quantitative info on substructures

Chlorpyrifos – DNT study design

Title: Anon. Developmental neurotoxicity study of chlorpyrifos administered orally via gavage to Crl:CD®BR VAF/Plus® presumed pregnant rats. 1998

Sponsor: Dow AgroSciences

Design:

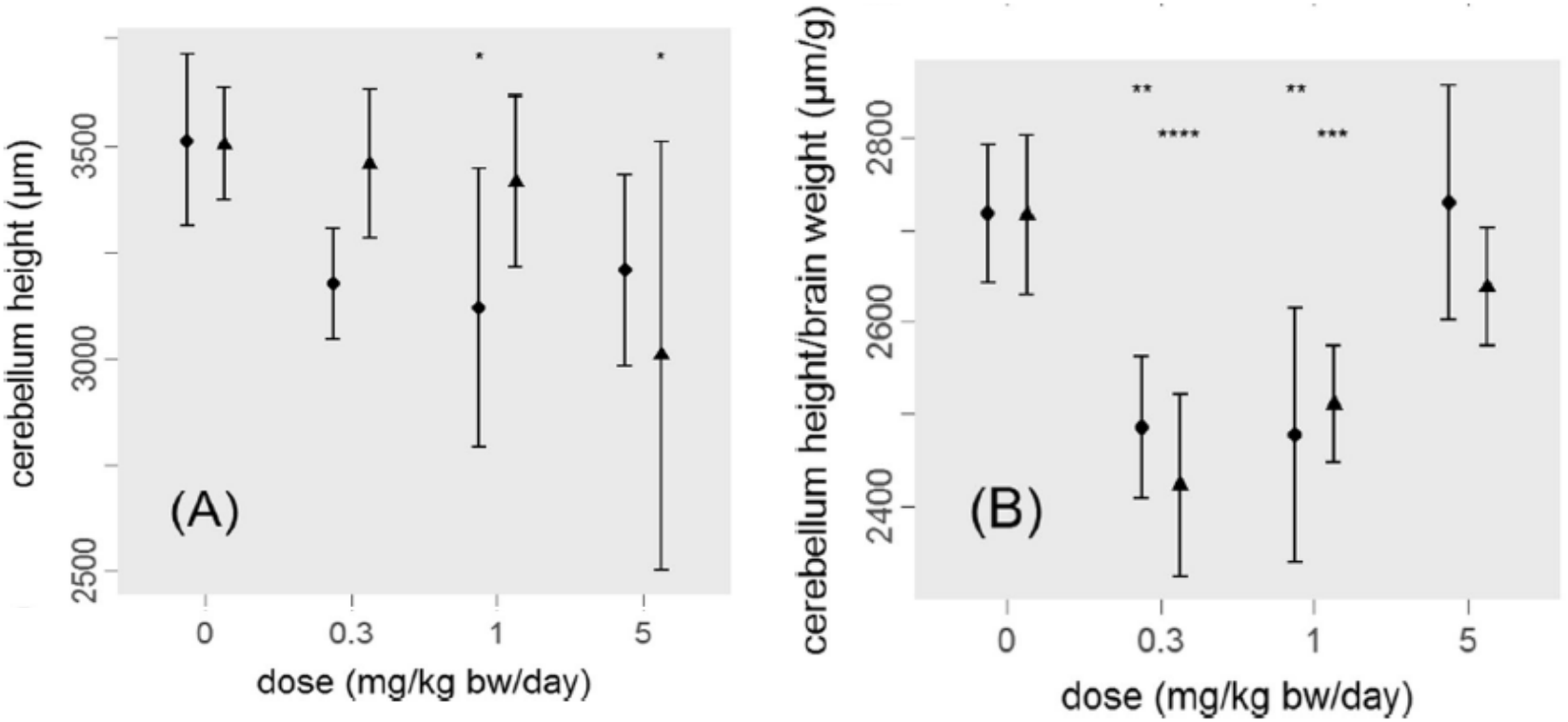
- **Number of animals**: 20 litters/dose
- **Exposure**: 0, 0.3, 1, 5 mg/kg/day by gavage to dam GD 6 – PND 11
- **Outcomes in offspring**: General toxicity, developmental landmarks, neuropathology/morphometrics (PND 11 + 65), neurobehaviour (motor activity, auditory startle, learning + memory/Biel maze)

Following slides:

1. Unreported effect, chlorpyrifos
2. Study design issues, chlorpyrifos
3. Chlorpyrifos-methyl

No full re-evaluation of studies.

Chlorpyrifos – cerebellum height PND 11 pups



Cerebellum height in relation to chlorpyrifos exposure, PND 11 pups. 6 animals/sex/dose group. ● females, ▲males. Means ± standard deviation.

(a) cerebellum height, (b) cerebellum height relative to brain weight.

Asterisks denote statistical significance in Dunnett's test:
* $p < 0.05$, **$p < 0.01$. ***$p < 0.001$, ****$p < 0.0001$ compared to control, posthoc to significant ($p < 0.05$) one-way ANOVA separately for sexes

No effect in PND 65 cerebellar height. Exposure limited in time, no info on substructures

Chlorpyrifos – PND 11 cerebellum height

Summary Reanalysis of Morphometric Data (Supplement 2 to DNT report, p. 9)
"Overall, high-dose male and female pups had smaller morphometric values than controls (Table 1). Because of the smaller absolute brain weights, the averages of the nine brain measurements were comparable to brain weight. (…) No effects attributed to treatment were noted in the mid- or low dose groups."

Table 1: Pup day-12 brain weights and linear measurements expressed as percent of control.

	Males				Females			
Variable	ANOVA	Low (%)	Mid (%)	High (%)	ANOVA	Low (%)	Mid (%)	High (%)
Brain weight	Significant	110.6	105.5	**88.5***	Significant	98.9	97.6	**91.3***
AP cerebrum	Significant	107.1	104.7	93.8	Not Sig.	102.4	103.2	98.0
AP cerebellum	Significant	105.6	102.0	**75.5***	Not Sig.	95.3	103.7	94.3
Frontal cortex	Not Sig.	100.9	100.3	94.4	Not Sig.	100.9	98.5	99.4
Parietal cortex	Significant	108.4	108.4	94.0	Not. Sig.	99.7	99.1	94.5
Caudate/putamen	Not Sig.	100.0	103.2	99.3	Significant	93.3	96.0	**90.3***
Corpus callosum	Not Sig.	103.4	99.0	100.0	Not Sig.	93.2	99.0	89.1
Hippo gyrus	Significant	111.1	107.5	91.2	Not Sig.	97.4	99.6	88.5
Cerebellum ht.	Significant	98.6	97.5	**85.8***	Significant	90.4	**88.8***	91.3
Ext germinal layer	Not Sig.	103.1	107.6	101.3	Not Sig.	94.0	106.5	105.6
Average		104.2	103.4	92.8		96.3	99.4	94.5

Statistical analyses were performed on data before conversion to % of control. ANOVA, if significant, was followed by Dunnett's. * = statistically significant by Dunnett's. Sig. = statistically significant, alpha = 0.05.

U.S. EPA (2000):
"These comparisons, however, are an inappropriate and inconclusive manipulation of the data, since a numerical value derived from averaging the relative values for all external and internal morphometric measurements is not meaningful. Such a derived number would not evaluate the differences between alterations in growth patterns or disruptions in discrete areas of the brain, which could be differentially altered as an adverse consequence of treatment."

No correction requested.

Chlorpyrifos – Study design issue 1. Positive controls

Positive controls are required for demonstrating the proficiency of the lab to perform DNT (and other) studies.

No neurodevelopmental outcome has a valid positive control.

Inability of the lab/test procedure to detect DNT of lead.

Chlorpyrifos – Study design issue 2. Exposure

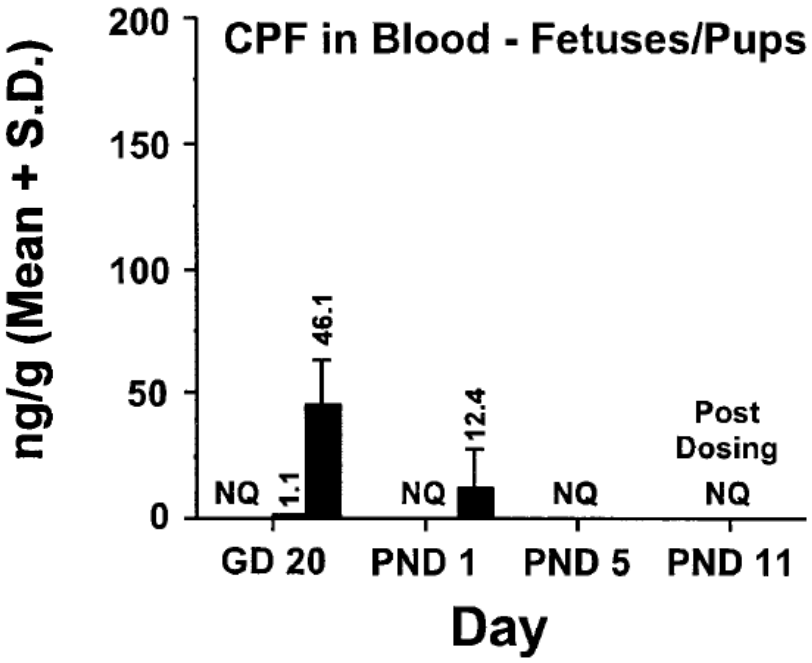
Brain development events have different timing relative to birth in different species.

Rats: peak of brain growth spurt approx. PND 11
Humans: peak of brain growth spurt around birth

Most relevant exposure in rat pups PND 0 – 11: internal dose equivalent to continued *in utero* exposure, i.e. blood concentration approx. constant before/after birth.

Chlorpyrifos – Study design issue 2. Exposure (continued)

Satellite study by Dow (Mattsson 2000) [1]



Limit of quantification 0,7 ng/g

Black bar – high dose (5 mg/kg/day)

Limited data suggest that exposure of nursing pups in DNT study is far below adequate levels during the most intensive period of brain development

[1] Mattsson 2000 www.ncbi.nlm.nih.gov/pubmed/10696792
See also Marty 2007 www.ncbi.nlm.nih.gov/pubmed/17928393

Chlorpyrifos – Study design issue 3. Post-hoc changes to statistical protocoll

Several slight changes to the evaluation of behavioural effects (motor activity, auditory startle, learning + memory) without clear justification introduced between draft and final report.

Consequences not spelled out - implications unclear – laborious to investigate.

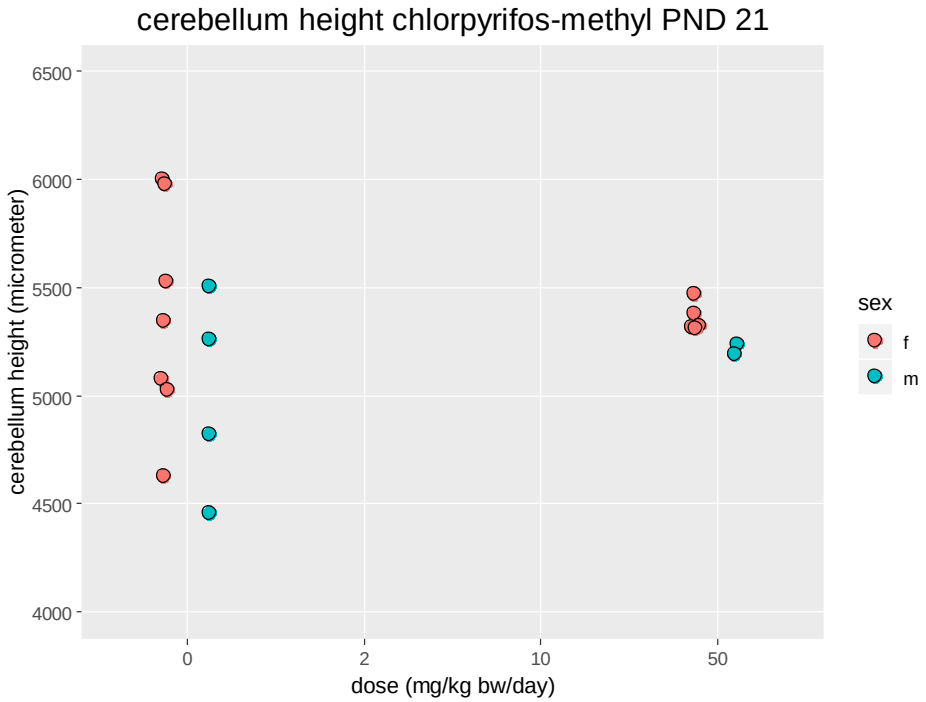
Chlorpyrifos – Study design issue 4. Type 2 error rate

α=0,02 for most of the study, without justification.

Chlorpyrifos-methyl – DNT study design

- **Title:** Anon. A Dietary Developmental Neurotoxicity Study of Chlorpyrifos-Methyl in Rats; 2015.
- **Guideline:** OECD TG 426 (2007). OECD guideline for the testing of chemicals. Developmental Neurotoxicity Study
- **Sponsor:** Dow AgroSciences
- **Number of animals**: 25 litters/dose
- **Exposure**: 0, 2, 10, 50 mg/kg/day dam dietary GD 6 – PND 21
- **Outcomes in offspring**: General toxicity, developmental landmarks, neuropathology/morphometrics (PND 21, 72), neurobehaviour (motor activity, auditory startle, learning & memory/Biel maze)

Chlorpyrifos-methyl – cerebellum height, PND 21



Planned: n=10/sex/group (control + high dose)

Lack of data points not highlighted in DNT report summary.

No reason for missing data given in DNT report. -> letter to the editor (March 2019): Absent points due to “non-homologous brain sections”)

Unusually narrow distribution of data points at high dose.

Absence of effect of chlorpyrifos-methyl on cerebellum height not established.

Chlorpyrifos-methyl – postnatal exposure of pups

- "Based on the results of the range-finding study [redacted] the F1 animals in the present study were exposed to the test substance *in utero*, as well as via the milk while nursing and via direct consumption of the diet during the latter portion of the lactation period." (DNT report, p. 42)
- Range-finding study apparently not submitted to EFSA.
- No pharmacokinetics provided in DNT study report, in spite of guideline requirements.
- Not possible to judge if exposure during nursing was adequate.

Implications – funding of studies

- Potential existance of funding bias indicated
- Difficult task for agency reviewers to find incorrect analyses – too voluminous.
- Single incorrectly reported tox study may have wide-ranging public health consequences (chlorpyrifos).
- Magnitude of problem unknown to us
- One option to avoid perceived/real funding bias:
  funding by company vs regulatory authority
- Regulatory attention needed

Implications – independent scientific studies in safety evaluation of pesticides

- Full access to complete tox study reports including raw data, methods, analyses, conclusions etc for researchers/public
- All types of studies must be carefully evaluated and included in regulatory risk assessment, considering their strengths and weaknesses. Here: epidemiological studies of DNT of chlorpyrifos/organophosphates
- Enhance use of academic studies in regulatory risk assessment e.g. using Science in Risk Assessment and Policy (SciRAP) approach
- Increase data availability in academic science while balancing other legitimate interests (e.g. privacy in human subjects research) – Transparency and Openness (TOP) standards