



Chlorpyrifos

Weight of Evidence Fails to Support Listing Under Proposition 65

DART IC Meeting
20 November 2008

11/20/08

Agenda

- ***Standard for Listing Under Proposition 65***
 - Christian Volz
McKenna Long & Aldridge LLP
- ***Evaluation of Epidemiology Data***
 - Carol J. Burns, MPH, PhD
The Dow Chemical Company
- ***Evaluation of Animal Data***
 - Daland R. Juberg, PhD
Dow AgroSciences

Standard for Listing Under Proposition 65

Statute:

“A chemical is known ... to cause ... reproductive toxicity ... if in the opinion of the state’s qualified experts it has been *clearly shown* through *scientifically valid testing* according to *generally accepted principles* to cause ... reproductive toxicity.”

Standard for Listing Under Proposition 65 (cont'd)

Duty of DARTIC:

“Render an opinion ... as to whether specific chemicals have been *clearly shown*, through *scientifically valid testing* according to *generally accepted principles*, to cause reproductive toxicity.”

Cal. Code Regs., tit. 22, § 12305(b)(1)

Guidance Criteria for Listing Chemicals as “Known to Cause Reproductive Toxicity”

General Principles:

- “In evaluating the sufficiency of data, a ***weight of evidence*** approach shall be used to evaluate the body of information available for a given chemical.” Guidance Criteria at 1.D.
- “In determining whether a chemical is to be ... listed as known ... to cause reproductive toxicity, the ***biological plausibility*** of the association between the adverse reproductive effects observed and the chemical in question should be considered. Confidence is increased when ... a sound scientific basis exists for the observed adverse effects and the known characteristics of the particular chemical. Conversely, confidence is decreased if the observed adverse effects are contradictory to the known characteristics of the particular chemical.” Guidance Criteria at 4.B.

Guidance Criteria: Human Studies

“Sufficient evidence in humans,” in the case of epidemiology studies, means studies that:

- provide **convincing evidence** to support a **causal relationship** between exposure to the chemical in question and the ... effect in question.
- This requires **accurate exposure** and toxicity endpoint classification and proper control of **confounding factors, bias**, and endpoint modifiers.

Guidance Criteria at 3.A.(1)

Guidance Criteria: Human Studies

(cont'd)

Weight of Evidence Considerations

- In general, “effects should occur in ***more than one human study*** for a chemical to be recommended for listing on the basis of epidemiologic evidence alone.
- A single well conducted epidemiologic study showing a ***clear relationship between exposure and effect*** may be sufficient to support listing provided ***there are not equally well conducted studies which do not show an effect*** and which call into question the repeatability of the observed effect in the “positive” study.
- Where epidemiology data are only “limited” or “suggestive,” a listing must be supported by “sufficient experimental animal data.”

Guidance Criteria at 3.A.(3), 3.B.

Guidance Criteria: Animal Studies

Whether animal studies are “sufficient evidence” to support extrapolation to humans, in most cases, is based on the following:

- The **experimental design** and presence of appropriate controls
- The exposure, in terms of **route of administration**, is relevant to expected human exposures
- The number of dose levels, so that the presence of a **dose-response relationship** can be evaluated
- *Consideration of **maternal and systemic toxicity***

Guidance Criteria at 3.C.(1)-(4)

Standard for listing under Prop 65 (cont.)

- Developmental toxicity
 - Proposition 65 regulates developmental effects caused by pre-natal exposures but not post-natal exposures. (OEHHA General Counsel William Soo Hoo. DART Committee meeting. 1996.)

Chlorpyrifos and Epidemiology

Carol J. Burns, MPH, PhD
November 20, 2008

Studies in humans

- 1A. Epidemiology studies specific to chlorpyrifos
 - 3 cohort studies (detail to follow)
 - Rull (no significant elevation)
- 1B. Publications not specific to chlorpyrifos
 - Samarawickrema (BuChE only)
 - Wolff (DEP and DAP)
 - Engel (DAP)
 - Young (DAP)
 - Serles Nielsen (PON1) no significant finding of pest treatment

Studies in humans

- 2. Clinical cases
 - Sherman – no consistent pattern to the anomalies
 - Sebe – attempted suicide, in utero fetal death

Standard for listing in humans

- 3. Weight of evidence.
 - a. ...effects should occur in more than one human study...
 - b. Data from a single...study...may be sufficient...provided there are not equally well conducted studies which do not show an effect.

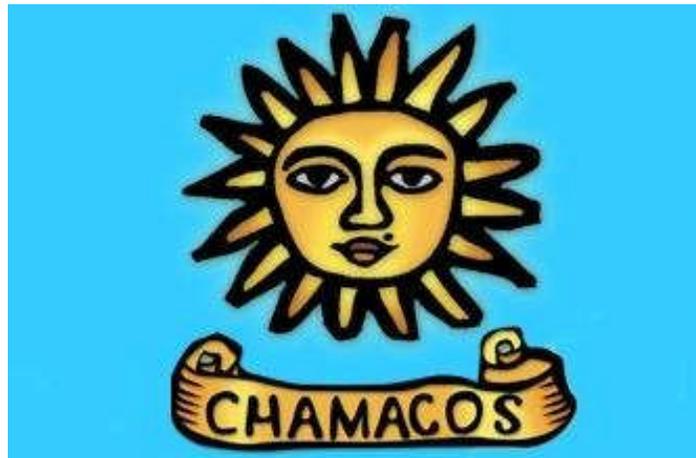
Story of 3 cohorts

- CHAMACOS (N~400)
 - Center for the Health Assessment of Mothers and Children of Salinas
 - ~ 40 publications
 - Lead investigator: Eskenazi
- Mt. Sinai (N ~ 400)
 - Lead investigators: Berkowitz, Wolff
- CCCEH (N~350)
 - Columbia Center for Children's Environmental Health
 - Lead investigators: Whyatt, Perera, Rauh
 - ~60 publications

Salinas Valley Birth Cohort

~40 publications

The CHAMACOS Center works with families in a farmworker community to learn how pesticides and other environmental exposures affect the health of mothers and children. CHAMACOS means small child in Mexican Spanish. The CHAMACOS Center is comprised of several research projects investigating the environment and children's health in the Salinas Valley, Monterey County, California.



Strengths

- Study focused on pesticides
- Robust study conduct
 - Used bicultural interviewers
 - Interviewed subjects 2X during pregnancy and collected spot urines
 - Bayley scales administered by trained psychometricians
- Used specific urine metabolite (TCPy)
 - Blood method used in CCCEH has been changed by CDC
 - Able to compare exposure to other studies

Limitations

- Participants not representative of all Californians
 - 85% Mexican born
 - 28% were field workers
- *In utero* exposure undocumented
- Exposure may have continued post birth

No consistency across studies

Birth Weight

Author, year	Exposure indicator	Birth weight
Eskenazi 2004	TCPy	+, No
CHAMACOS	Total DAP	+, No
Berkowitz 2004	TCPy	+, No
Mt. Sinai	Reported pesticide use	+, No
Whyatt 2004	Chlorpyrifos in cord blood	-, Yes
CCCEH	Personal air samples	-, No

- Birth weight **increases** with increasing TCPy, Total DAP, and reported pesticide use in CHAMACOS and Mt. Sinai.
- Birth weight **decreases** with increasing cord blood and personal air samples in CCCEH
- Only cord blood analysis is statistically significant.

+: increasing direction,
Yes: statistically significant

No consistency across studies

Birth Length

Author, year	Exposure indicator	Birth Length
Eskenazi 2004	TCPy	+, No
CHAMACOS	Total DAP	+, Yes
Berkowitz 2004	TCPy	+, No
Mt. Sinai	Reported pesticide use	+, No
Whyatt 2004	Chlorpyrifos in cord blood	-, Yes
CCCEH	Personal air samples	-, No

- Birth length **increases** with increasing TCPy, Total DAP, and reported pesticide use in CHAMACOS and Mt. Sinai.
- Birth length **decreases** with increasing cord blood and personal air samples in CCCEH
- Statistically significant are Total DAP and cord blood, in opposite directions.

+: increasing direction,
Yes: statistically significant

No consistency across studies

Head Circumference

Author, year	Exposure indicator	Head Circum.
Eskenazi 2004	TCPy	+, No
CHAMACOS	Total DAP	+, Yes
Berkowitz 2004	TCPy	=, No
Mt. Sinai	Reported pesticide use	-, No
Whyatt 2004	Chlorpyrifos cord blood	-, No
CCCEH	Personal air samples	-, No

- Head circumference **increases** with TCPy and Total DAP in CHAMACOS study.
- Mean head circumference is equal by TCPy in Mt. Sinai. PON1 results similar for < and > LOD.
- Head circumference **decreases** with increasing cord blood. These results are not statistically significant.

+: increasing direction,
Yes: statistically significant

No consistency across studies

Author, year	Exposure indicator	Study area	Birth weight	Birth Length	Head Circumference
Eskenazi 2004	TCPy	Salinas Valley, CA	+, No	+, No	+, No
	Total DAP		+, No	+, Yes	+, Yes
Berkowitz 2004	TCPy	Mt Sinai, NYC	+, No	+, No	=, No
	Reported pesticide use		+, No	+, No	-, No
Whyatt 2004	Chlorpyrifos in cord blood	Harlem, NYC (CCCEH)	-, Yes	-, Yes	-, No
	Personal air samples		-, No	-, No	-, No

+: increasing direction, Yes: statistically significant

No consistency across studies

Developmental Delay

Author, year	Sample size	Study area	Mental Development Index		
			12 mo	24 mo	36 mo
Eskenazi, 2007	372 (24 mo)	Salinas Valley, CA	No	No	*
Rauh, 2006	228 (36 mo)	Harlem, NYC (CCCEH)	No R² = 0.04	No R² = 0.15	No R² = 0.25

* Testing not done; No: Not statistically significant, R² not reported in Eskenazi

No consistency across studies

Developmental Delay

Author, year	Sample size	Study area	Physical Development Index		
			12 mo	24 mo	36 mo
Eskenazi, 2007	372 (24 mo)	Salinas Valley, CA	No	No	*
Rauh, 2006	228 (36 mo)	Harlem, NYC (CCCEH)	No $R^2 = 0.02$	No $R^2 = 0.04$	Yes $R^2 = 0.11$

*Testing not done; R^2 not reported in Eskenazi
Model included ETS, Chlorpyrifos, Race, Gender, Age, IQ, Education, HOME

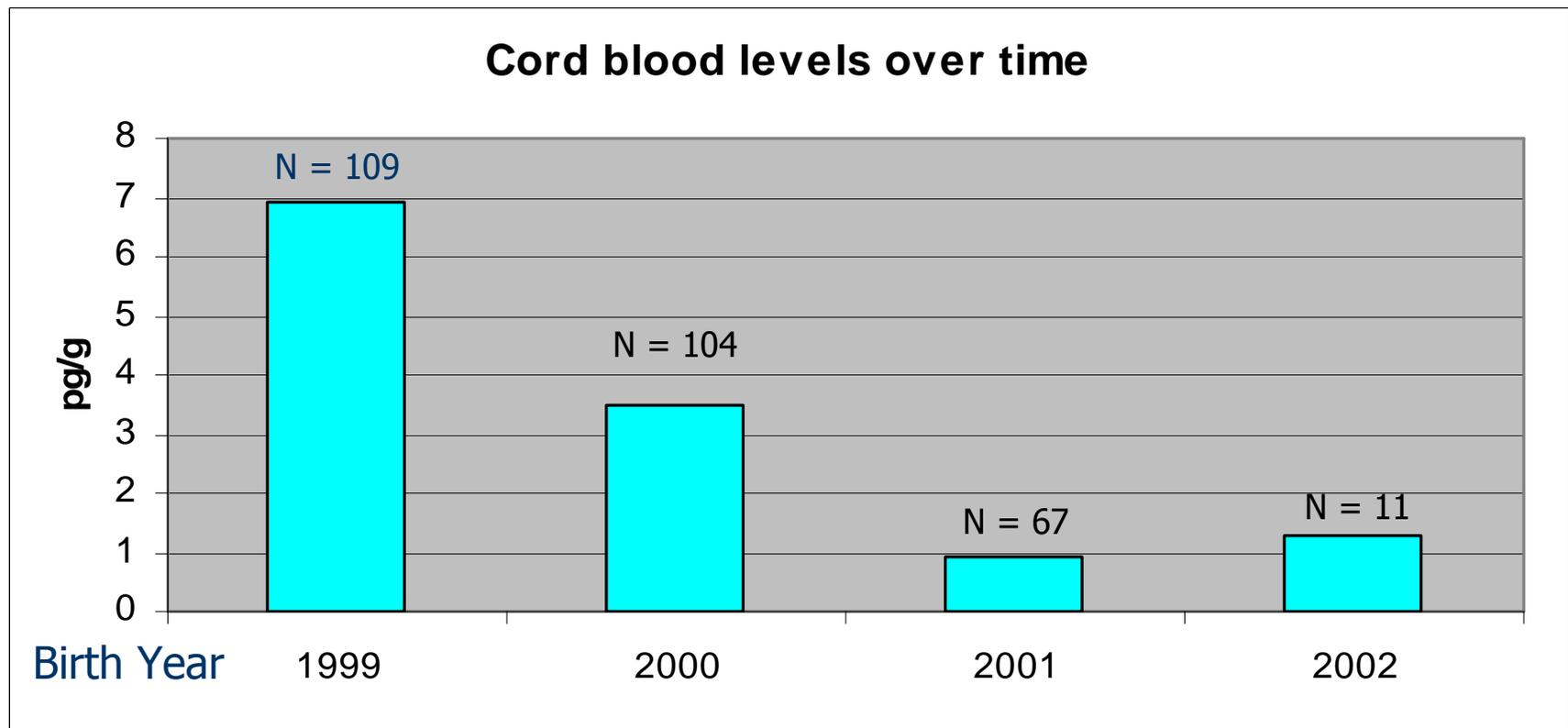
Results over time

Reported by CCCEH

- Residential use withdrawn in 2001
- Cord blood levels reduced by more than 80% in CCCEH
- Question: Does health improve after removal of exposure source?

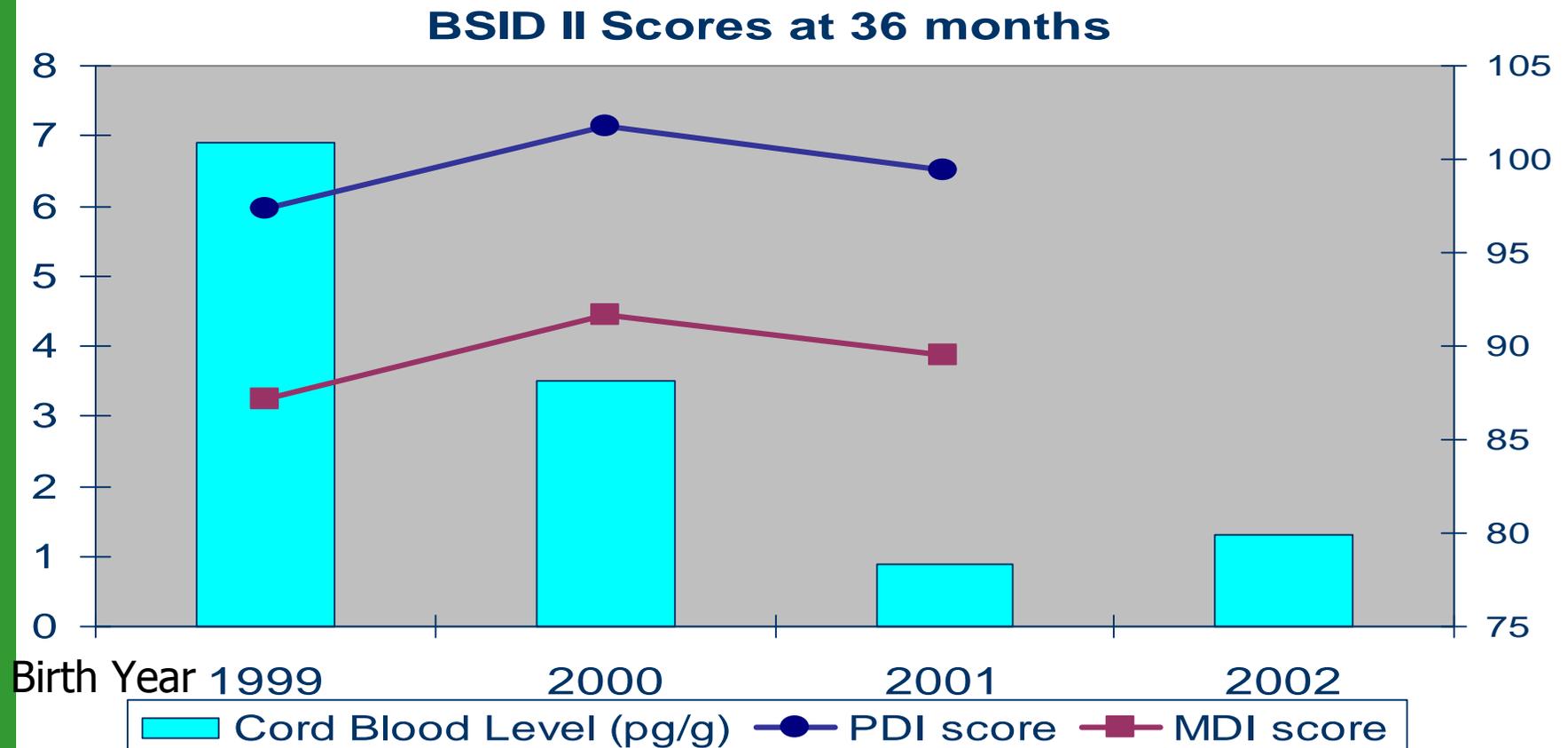
Cord blood levels decline

Whyatt et al. Toxicol Appl Pharmacol 2005;246-254



Exposure declines – Scores don't improve

Rauh et al. Pediatrics 2006;118:1845-1859)



PDI: $P=0.06(1999-2000)$; $P>0.05(2000-2001)$;
MDI: $P=0.02(1999-2000)$; $P>0.05(2000-2001)$

Biological Plausibility

- Long standing mode of action is through cholinesterase inhibition.
 - Most sensitive effect
 - Used as POD for risk assessment
- Chlorpyrifos levels estimated by blood and urine in human studies are too low to inhibit plasma butyryl cholinesterase.

Key points on epidemiology

- The 3 birth cohort studies are not consistent
 - CHAMACOS study, focused on pesticides, found no significant findings.
 - CCCEH is the only study to report statistically significant adverse effects.
- The *natural experiment* does not support a cause and effect
- Exposures are low and unlikely to inhibit cholinesterase

Weight of evidence criteria

- a. ...effects should occur in more than one human study.
- b. Data from a single...study...may be sufficient...provided there are not equally well conducted studies which do not show an effect.

Evidence is not sufficient

Effects do not occur in more than one human study.

There are equally well conducted studies which do not show an effect.



Questions?

Chlorpyrifos and Animal Studies

Daland R. Juberg, Ph.D.

November 20, 2008

Outline

- Background
- Developmental Toxicity
 - Guideline studies
 - Other studies in HID
 - Developmental neurotoxicity study
- Reproductive Toxicity
- Conclusions

Background

- Pesticide with extensive toxicological database
- Toxicological mode of action: cholinesterase (ChE) inhibition
- ChE inhibition (neurotoxicity) is the most sensitive effect used for risk assessment on a global basis

Key Developmental Toxicity Studies

- 4 studies in 3 animal species (rat, mouse, rabbit)
- Conducted according to US EPA Guidelines
 - Comprehensive
 - Scientifically valid
- Significant findings
 - No developmental toxicity in the absence of maternal toxicity
 - Little or no developmental toxicity even at doses that produced tremors, salivation and even death in dams
 - No teratogenicity in any species
 - Cholinesterase inhibition is the most sensitive endpoint

Mouse Developmental Toxicity Study (Deacon et al., 1980)

- Relative lack of developmental toxicity, even at maternally toxic doses
- 1 mg/kg/d: Maternal ChE inhibition, but no developmental toxicity
- 10 mg/kg/d: Maternal toxicity (e.g., tremors, salivation) but no developmental toxicity
- 25 mg/kg/d: Severe maternal toxicity, including death (4/47); decreased fetal body weight and delayed ossification

“C. Sufficient evidence in experimental animals...based on the adequacy of...”

- Experimental design
- Route of administration is relevant to expected human exposures
- Number of dose levels
- Consideration of maternal toxicity

Limitations of Other Developmental Toxicity Studies

- Many use sc or ip route of exposure
- Some involved postnatal exposure only
- Inadequate dose groups; data not fully reported
- Many involved DMSO which is known to exert neurotoxic effects of its own
- Numerous studies claim effects on offspring below threshold for maternal toxicity, but
 - Cholinesterase inhibition not often measured
 - Maternal body weight gain not reported

Developmental Neurotoxicity Study (Maurissen et al. 2000)

- Study in rats conforms to USEPA Guideline
- Prenatal and postnatal exposure
- No evidence of developmental toxicity in absence of maternal toxicity
 - No effects on birth weight except at maternally toxic top dose
 - No effects on learning and memory even at top dose
- “No evidence of selective developmental neurotoxicity following exposure to chlorpyrifos.”

Reproductive Toxicity

- “No effects on the reproductive or fertility indices or on the histopathology of reproductive tissues were observed in animals at dose levels that resulted in significant cholinesterase inhibition. Severe testicular damage resulting in reduction in sperm count and fertility was noted in a study at higher dose levels” – HID
- The one study (Joshi *et al.*, 2007) referred to has multiple problems and none of its reported results have been confirmed by others
 - High exposures above ChEI
 - Toxicity at all dose levels, not well-characterized
 - Mating data not included

Reproductive Toxicity (cont'd)

- Multigeneration study (Breslin *et al.*, 1996) indicated no effect on fertility or histopathology of reproductive organs at the highest dose
- No neonatal effects in absence of maternal toxicity
- No evidence of reproductive structures as a target organ in other long-term studies
- Other studies at lower dose levels (Thompson *et al.*, Dietz *et al.*, James *et al.*) confirm absence of reproductive effects

Conclusions

- Studies representing “scientifically valid testing according to generally accepted principles” do not indicate developmental toxicity across a number of species in the absence of maternal toxicity.
- Consistent evidence across studies demonstrates fetuses to be less sensitive than dams.
- The weight of the scientific evidence does not demonstrate that chlorpyrifos produces male or female reproductive toxicity in animal studies.
- The scientific data indicate that chlorpyrifos has not been clearly shown to cause developmental or reproductive toxicity.

Summary

Chlorpyrifos has not been clearly shown through scientifically valid testing according to generally accepted principles to cause developmental, female reproductive, or male reproductive effects.

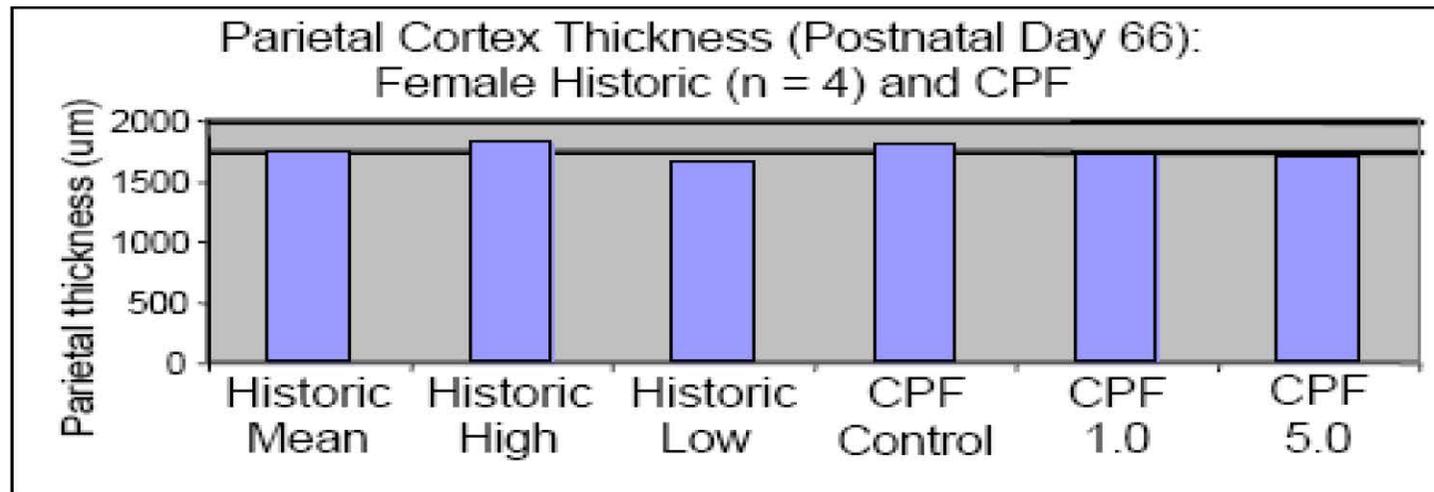
- No sufficient evidence in humans
- No sufficient evidence in experimental animals



Questions?

Additional slide

Data from Hoberman and Garman, Supplement 3, 10/9/2000.



Female Day 66	Parietal Thickness (um)	% historic mean	% control mean
Hist mean	1738	100.0	97.0
Hist high	1824	104.9	101.8
Hist low	1656	95.3	92.4
CPF Cont	1792	103.1	100.0
1 mg/kg/d	1716	98.7	95.8
5 mg/kg/d	1700	97.8	94.9