FC Toxicity/Safety Testing

In Particular

PFOS & N-EtFOSE

Toxicology work being done and/or coordinated by Deanna Nabbefeld, Andrew Seacat, Paul Lieder, Mike McNamara, Marv Case, John Butenhoff

Analytical work being done by Kris Hansen and co-workers at Environmental Analytical laboratory (BLDG 2) as well as Fred De Roos in Central Research (BLDG 201)

Introductory Remarks

I am a

veterinary pathologist

working toxicologist

in Corporate Toxicology

Multi-facetted situation – today will be talking about one aspect of it

Toxicology deals with

biological variation

animal to man extrapolation

data that sometimes can have more than one possible interpretation

risk assessments/judgements

Remember

Toxicology and Safety are the two edges of the **same** sword

Proving absolute safety is impossible – can not prove a negative

No black and white answers – all relative

EPA/HPV

HPV = High Production Volume chemicals

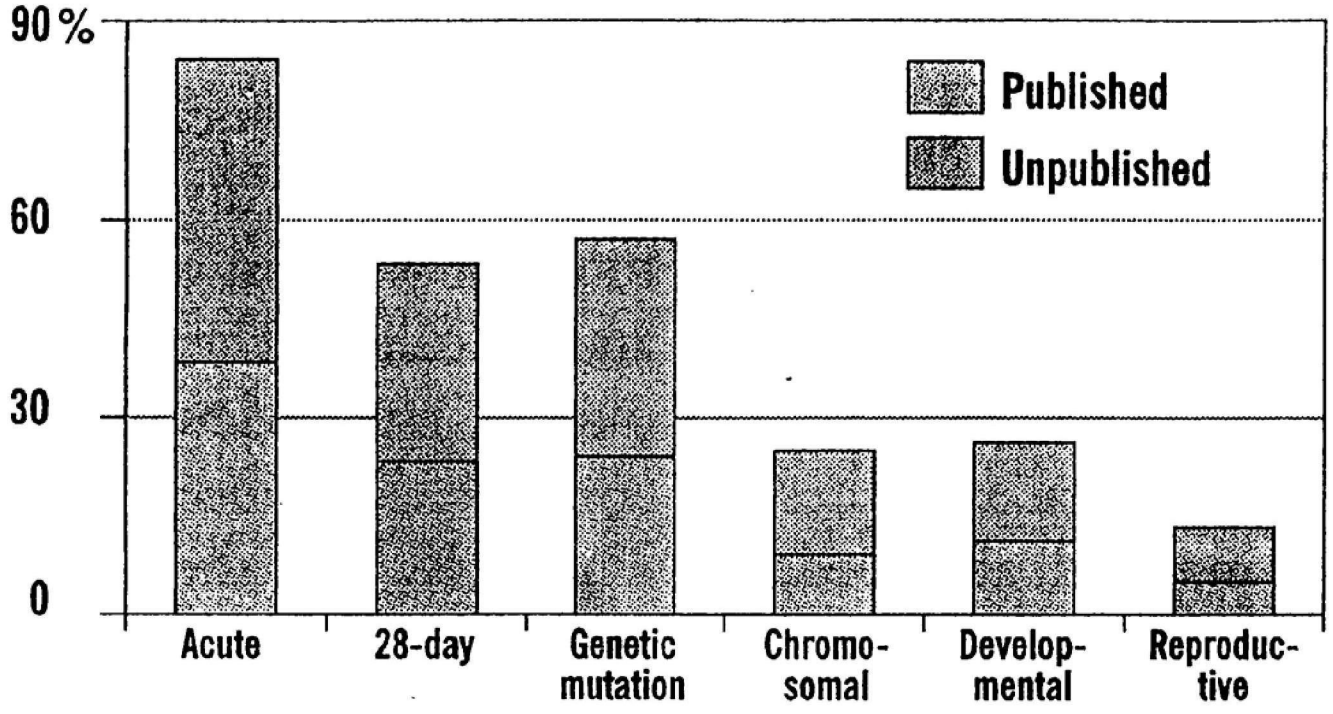
EPA wants each HPV evaluated for six toxicity endopoints

Called SIDS (Screening Information Data Set)

EPA plans to issue TSCA rule mandating SIDS testing if chemical companies fails to do testing voluntarily

Two FC that would be HPV are PFOS and N-EtFOSE

OPENING THE FILES*



* Percentage of high production volume (HPV) chemicals for which human toxicity data is publicly available and for which companies are known to possess unpublished data. Excludes 275 HPVs nominated for testing under the screening information data sets program. Source: CMA.

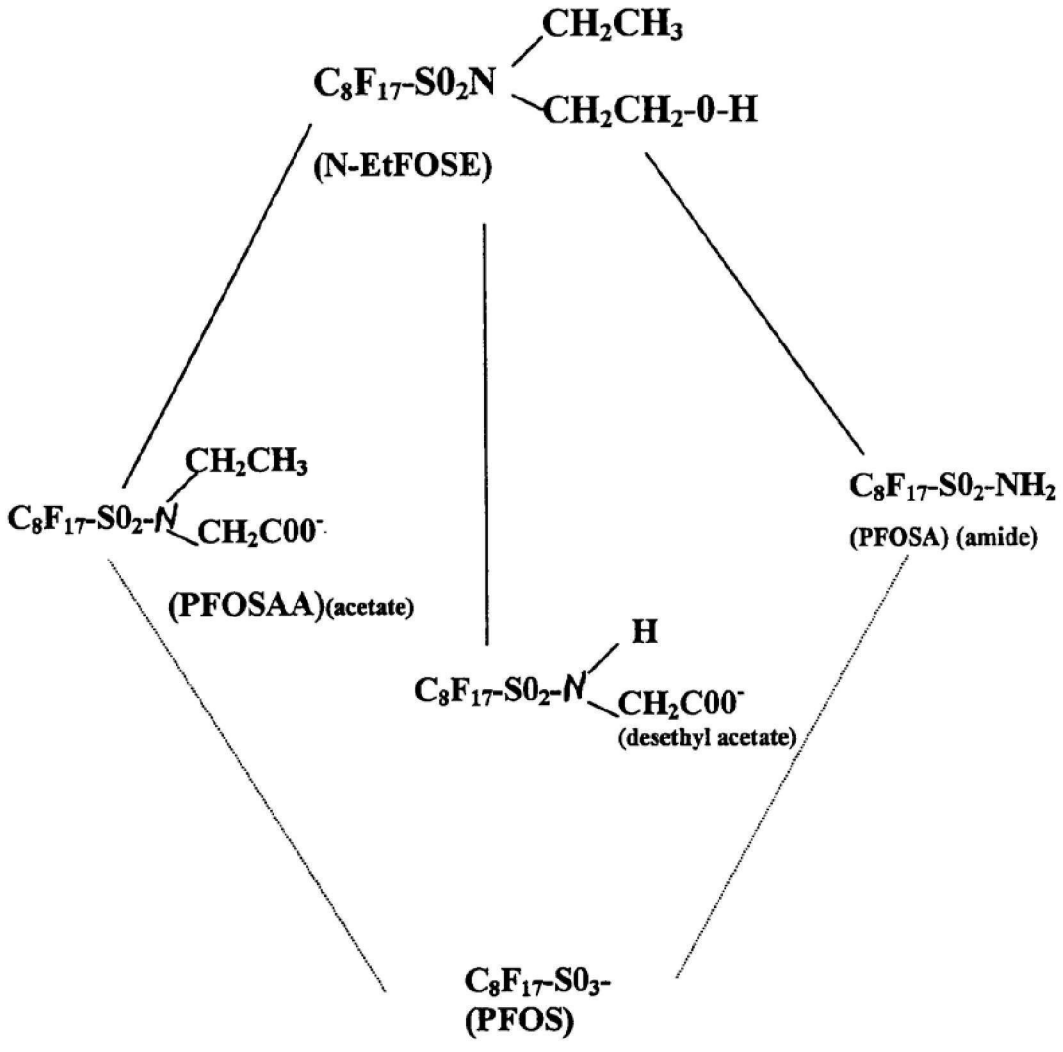
BIT OF HISTORY

In 1968, Taves published results describing organic fluorine which was bound to serum albumin in human blood samples

In early 1980's select 3M employees monitoring began

No health effects associated with FC exposure

1994-95 improved analytical technology, LCMS, applied



Rat Carcinogenicity Studies

Two-year Oral (feeding) Rat Studies on N-EtFOSE (FC 10) & PFOS (FC 95)

N-EtFOSE – dosing started 26 January 1998; final necropsy Jan/Feb 2000; histopathology of tissues Aug/Sep 2000

PFOS – dosing started 20 April 1998; final necropsy late April 2000; histopathology of tissues Nov/Dec 2000

Main objectives – 1) determine carcinogenicity potential of compounds
2) determine NOEL for chronic toxicity

Secondary objective – determine subchronic toxicity; have extra animals for interim necropsies

Complex studies

compound mixed in diet

interim necropies at 4, 14 & 52 weeks with mechanistic measurements

recovery group at high dose

lots of pathology data – approximately 10,000 tissue slides to be prepared and examined microscopically

George Moore

Hormone Effects?

Rat Carcinogenicity Studies

14-Week Interim Necropsy Results

	N-EtFOSE	PFOS
Body Weights	dose related ↓ ♂ & ♀	↓ high dose ♂ & ♀
Blood Counts	no effect	no effect
Blood Chemistry	↓ glucose high ♂ & ♀ ↓ cholesterol dose related ♂ & ♀ ↑ Pal-CoA oxidase	no glucose effect ↓ cholesterol high ♂ ↑ Pal-CoA oxidase liver enzyme
Liver Weights	↑ dose related ♂ & ♀	↑ high dose ♂ & ♀ ∴ liver target organ
Liver Pathology	enlarged, vacuolated liver cells	enlarged, vacuolated liver cells

Scheme of the pathways of long-chain fatty acid transport and metabolism in the hepatocyte showing the potential function of L-FABP in the binding and intracellular diffusion of fatty acids and fatty acyl-CoA. ACS, acyl-CoA synthase; C_8FA-CoA, octanocyl-CoA; CM, chylomicron; FA, long-chain fatty acid; FA-CoA, long-chain fatty acyl-CoA; HL, hepatic lipase; LPM-FABP, liver plasma membrane FABP;

Rat Carcinogenicity Studies

Peroxisome Proliferators

Peroxisomes are cytoplasmic organelles that function in cell respiration, energy metabolism, and fatty acid metabolism. They are present in most body cells but most common in liver and kidney.

Known peroxisome proliferators include hypocholesterolemic pharmaceuticals (clofibrate), herbicides (2,4-D), plasticizers (phthalates).

FC effects seen at 14 weeks are typical of peroxisome proliferators

These include:

- ↓ blood cholesterol
- ↑ liver size
- ↑ Pal-CoA oxidase
- swollen liver cells with vacuoles in cytoplasm
  → increased # of peroxisomes?

Peroxisome proliferation is a rodent effect; not known to occur in monkeys or man.

In rodent carcinogenicity studies, peroxisome proliferators have been related to increased numbers of benign tumors in the liver, pancreas and testis.

Final proof that compound is peroxisome proliferator is electron microscopic examination of liver of treated animals

Proceeding with an electron microscopy study with select FC compounds; working on final protocol

Tiers: peroxisome means to man?
chol. reduction → a drug?

Mechanism of Toxicity Efforts

Bioenergetics

Work being done under research contact with Ken Wallace, U of Minn School of Medicine, Duluth

Have determined that perfluoroacid and derivative compounds interfere with bioenergetics (rat liver mitochrondria in vitro data)

Various mechanisms of energy uncoupling (metabolic disturbance of ATP production) appear to be involved

Compounds varied in potency

Future work to compare effects on liver mitrochrondria of different species – rat, guinea pig, monkey, man.

Mechanism of Toxicity Efforts

Binding to Carrier Proteins & Cellular Membranes

Work being done by Andrew Seacat and Deanna Nabbefeld in Strategic Alternative Toxicology Laboratory, BLDG 270.

Have shown that FC compounds can bind to rat liver fatty acid binding protein (L-FABP)

Future work to

Compare binding of various species L-FABP – rat, guinea pig, human.

Determine L-FABP binding of different FC chain length

Investigate where in cell FC molecules are located.

Six-Month Monkey PFOS Toxicity Study

Dosing started 26 August 1998

Four males & four females in each dose group – control, low, mid & high dose with two/sex additional animals for 3-month recovery in control, mid & high dose groups

Dose levels are 0, 0.03, 0.15 & 0.75 mg/kg/day

The low dose of 0.03 mg/kg/day is expected to be a no-effect-level

The mid dose of 0.15 mg/kg/day a possible no-adverse-effect-level

The high dose of 0.75 mg/kg/day should produce mild toxicity

Six-Month Monkey study results (2 month)

Clinical signs of toxicity	none
Body weight	no effect
Blood counts	no effect
Blood chemistry	possible slight ↓ cholesterol

Reproduction Studies

Study Outlines

Two-generation Reproduction

Study Objectives: determine whether compound has adverse effect on reproductive functions and on development of second generation including its reproduction function

Study Procedures:

dose males & females rats 4 to 6 weeks before mating

mate and dosing continues during pregnancy

delivery F_1 pups and continue dosing during lactation

wean and then dose F_1 pups during growth

at sexual maturity mate F_1 pups and continue dosing during pregnancy and lactation

stop study at weaning of F_2 pups

Moore: Considered definitive study for endocrine disrupt. "Will Twinkies pass the test?"

Teratology

Study Objective: determine whether compound produces birth defects

Study Procedures:

dose pregnant females (rats & rabbits) only during pregnancy

take pups day before delivery

detailed internal & skeletal exam of pups

Teratology Study Results/Status

N-EtFOSE Rat Teratology

No teratogenic effect

N-EtFOSE Rabbit Teratology

No teratogenic effect

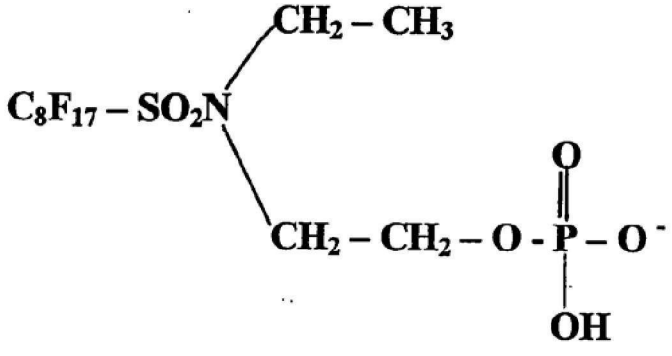
PFOS Rabbit Teratology

No teratogenic effect

PFOS Rat Teratology

No teratogenic effects found in 1981 3M study nor in published study from Haskell Laboratory (DuPont)

Monoester



Monoester – Metabolism & Absorption

Preliminary Rat Study Results

Liver Concentrations

	N-EtFOSE	PFOSAA (acetate)	PFOSA (amide)	PFOS
1.5 hrs after iv dose	620 ppb	1520 ppb	80 ppb	480 ppb
4 days after iv dose	---	230 ppb	300 ppb	>2000 ppb
28 days after iv dose	---	---	---	>2000 ppb

Tiers: held in liver?
what's held back is of greatest concern?
PFOS not rec. bound by fatty acid.
Case: where in liver held?

IOA

(iso-octyl acrylate)

Another HPV - SIDS test information complete

How compare to 2-Ethyl Hexyl Acrylate? (don't know))

Acute – dermal

Relatively non toxic Tom - OECS operations - competitiveness

Vesley Fran Brown published info on 2 EHA

Genetic toxicity (gene mutation) – Ames, yeast re-combinant

Genetic toxicity (chromosomal) – mouse lymphoma, cell transformation

Repeat dose toxicity – dermal mouse

Vesley move on 3M to to 2EHA!

case - where there - safety impt.

Reproductive toxicity – dermal rat

Lockwood Carlson – protocols accepted by Govt?
(yes

Newmark - Compare 3M vs DuPont
Wide - yes

Re-Invention Teams

Paul Lieder and Andrew Seacat - toxicologists working with teams

Developed 28-day rat toxicity screening test protocol

> Worked with Barbara Nelson in Procurement Operations and obtained a cost of $15,000 per compound (one dose level)

Developed 5-day inhalation toxicity screening protocol; recently obtained cost of ≈ $15,000 per compound

First oral study on re-invention materials dosing started 4 October 1998

Andrew, Paul, and Deanna – Strategic Toxicity Testing lab in 270

Nowak - what about testing actual products?

Tiers - Test impure stuff first? then go to pure?

Moore - doing NEXPOSE relieves duty to do ~~[illegible]~~ other? maybe

Clemens - (-) effect in lit. have to do more.