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 endpoints

Called SIDS (Screening Information Data Set)

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

Two FC that would be HPV are PFOS and N-EtFOSE
* 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volume (HPV) chemicals for which human toxicity data is publicly available and for which companies are known to possess unpublished data.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)


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 necropsies 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

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Rat Carcinogenicity Studies
14-Week Interim Necropsy Results

<table>
<thead>
<tr>
<th></th>
<th>N-EtFOSE</th>
<th>PFOS</th>
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<tbody>
<tr>
<td><strong>Body Weights</strong></td>
<td>dose related ↓</td>
<td>↓ high dose ♂ &amp; ♀</td>
</tr>
<tr>
<td></td>
<td>♂ &amp; ♀</td>
<td></td>
</tr>
<tr>
<td><strong>Blood Counts</strong></td>
<td>no effect</td>
<td>no effect</td>
</tr>
<tr>
<td><strong>Blood Chemistry</strong></td>
<td>↓ glucose high ♂ &amp; ♀</td>
<td>no glucose effect</td>
</tr>
<tr>
<td></td>
<td>↓ cholesterol dose related ♂ &amp; ♀</td>
<td>↓ cholesterol high ♂</td>
</tr>
<tr>
<td></td>
<td>↑ Pal-CoA oxidase</td>
<td>↑ Pal-CoA oxidase</td>
</tr>
<tr>
<td><strong>Liver Weights</strong></td>
<td>↑ dose related ♂ &amp; ♀</td>
<td>↑ high dose ♂ &amp; ♀</td>
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<td></td>
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</tr>
<tr>
<td><strong>Liver Pathology</strong></td>
<td>enlarged, vacuolated</td>
<td>enlarged, vacuolated</td>
</tr>
<tr>
<td></td>
<td>liver cells</td>
<td>liver cells</td>
</tr>
</tbody>
</table>

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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

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.

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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 mitochondria 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 mitochondria 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 F1 pups and continue dosing during lactation
- wean and then dose F1 pups during growth
- at sexual maturity mate F1 pups and continue dosing during pregnancy and lactation
- stop study at weaning of F2 pups

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

<table>
<thead>
<tr>
<th></th>
<th>N-EtFOSE</th>
<th>PFOSAA (acetate)</th>
<th>PFOSA (amide)</th>
<th>PFOS</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.5 hrs after iv dose</td>
<td>620 ppb</td>
<td>1520 ppb</td>
<td>80 ppb</td>
<td>480 ppb</td>
</tr>
<tr>
<td>4 days after iv dose</td>
<td>---</td>
<td>230 ppb</td>
<td>300 ppb</td>
<td>&gt;2000 ppb</td>
</tr>
<tr>
<td>28 days after iv dose</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>&gt;2000 ppb</td>
</tr>
</tbody>
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Tired: held in liver?
what is held best is of greatest concern?
PFOs not necessarily bound by fatty acid.
Sure: what in liver held?
IOA
(iso-octyl acrylate)

Another HPV - SIDS test information complete

Acute - dermal

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

Genetic toxicity (chromosomal) - mouse lymphoma, cell transformation

Repeat dose toxicity - dermal mouse

Reproductive toxicity - dermal rat

Lockwood Carlson - protocols rejected by Court?

Newmark - compare 3M vs Defendant

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Subject to Protective Order in Palmer v. 3M, No. C2-04-6309

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2717.0020
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