Toxicity of Microcystin-LR and Anatoxin a

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Background

- Significant cyanobacterial blooms in England and Wales at the end of 1990s.
- Toxicity in domestic and farm animals from scums.
- Concern for public and for drinking water.
- Very few data on toxicity.

Next Steps

- Primary toxin identified was Microcystin-LR.
- Other Microcystins present but LR the most toxic.
- Some Anatoxin a also seen but quite rarely.
- Need for toxicity data.

Developing Toxicity Data

- To develop guidelines for recreation and drinking water need good data.
- That implies using pure compound so no interferences and unknowns.
- Studies carried out to GLP so equivalent to a regulatory submission for an industrial chemical.

Problems

- Not much pure compound available for either toxin.
- Need to design studies within the constraints of what was available.
- Much of data already available was from administration by intraperitoneal injection.

Approach to minimising quantity

- Use the mouse.
- Need oral data so oral gavage.
- 13 weeks for Microcystin-LR
- 28 days for Anatoxin a
- Developmental toxicity
- Also comparison of oral and IP acute toxicity in mouse and rat.

Experimental

- Mouse- Cr1:CD-1(ICR)BR strain (VAF plus)
- Rat- Cr1:CD(SD)BR(VAF plus)
- Material dosed in aqueous solution.

Acute Study M-LR

- 5 male and 5 female mice and rats oral gavage 500, 1580 or 5000 µg/kg bw.
- Mice only, IP at 50, 158 or 500 µ/kg bw.
- Approx LD50s and survivors observed for 14 days, necropsied and lung and liver histopath.

Acute result

- Mouse IP – LD50 between 50 and 158 µg/kg. All died at 158 none at 50. Steep curve.
- Mouse oral – about 5000 µg/kg. One F died at 1580, 2 M and 1 F at 5000.
- Rat oral – 1M died at 5000 so LD50 greater than 5000 µg/kg
- Pathology as expected.

13 week repeat dose study

- Dose ranging study over 14 days.
- Groups of 5 Male and 15 Female mice dosed by oral gavage daily for 13 weeks
- Vehicle control, 40, 200 or 1000 µg/kg.
- Clinical observation, food consumption & body weights. Eye exam. Blood in final week for haematology and biochemistry.
- High dose all tissues for histopathology plus lungs, liver and kidney from other groups plus any gross lesions at necropsy.

13 week results

- Top dose 1 F died and 1 M humanely killed.
- Body weights no dose response. Males all down cf controls but only 7%. F 29% increase in mean BW gain in mid dose.
- Haematology – slight but sig increase in top dose females in haemoglobin, RBC, PCV.
- Biochemistry – top dose high AP and transaminases and mid males transaminases. Male reduction in total protein and albumin sig at top two doses.

Histopathology

- Treatment related change only in liver.
- Predominantly top dose.
- Similar but less marked lesions in a few mid-dose.
- Nothing in control or low dose.

Conclusion

- Some uncertainty regarding significance of blood biochemistry except at top dose.
- Clear no adverse effect level of 40 µg/kg for histopathology.
- Effects at mid-dose were relatively minor and in few animals.

Developmental Toxicity

- Dose ranging with decision of doses of 0, 200, 600 and 2000 µg/kg.
- Groups of 26 time mated females dosed once daily days 6 to 15 of pregnancy
- Day 18 animals humanely killed and necropsied.
- Fetuses weighed, sexed and examined for external, visceral and skeletal abnormalities.

Developmental Results

- Maternal tox in top dose. 7 died and 2 humanely killed. Gross path signs of severe liver toxicity.
- Surviving females no adverse effects on body weight, food consumption or clinical condition. No evidence of embryolethality or teratogenicity or change in implantations or live fetuses. No treatment-related increases in incidence of abnormalities
- Embryotoxicity with reduced fetal bw and delayed ossification in top dose. Expected with maternal tox.
- Other groups nothing.
- NOAEL - 600 µg/kg bw

Conclusions

- No effects on fetal development.
- Acute studies confirm other data. Between 30 and 100 times less toxic by oral route cf IP. Some signs of species variation.
- Longer term sig reductions in BW and BW gain in males but within normal range for this strain. Redn in protein and albumin in males no dose response and within strain ref ranges.
- None of blood chemistry adequately mirrors that seen in pigs (Falconer et al 1994) but some trends similar so significance unclear.

Pathology and Overall Conclusions

- Lesions only in liver; generally slight and primarily in top dose.
- In view of ambiguity in liver function indicator results reasonable to use clear pathology NOAEL of 40 µg/kg as overall NOAEL.
- This is 400 times greater than the amount received by a 10 kg child drinking 1 litre containing 1 µg/litre (about 5000 cells/ml).
- This study reflects all dose at one time cf consumption over a day.

Anatoxin a

- Alkaloid neurotoxin probably through pharmacological action.
- Pharmacological investigation to determine if nicotinic agonist.
- Subacute toxicity and screening for teratogenicity.
- Even less material available!

Background

- Most commonly encountered in UK waters, previously VFDF (very fast death factor)
- Structural analogue of cocaine, potent neuromuscular blocking agent and interacts with neuronal nicotinic AC receptors paralysing respiratory muscles.

Experimental

- *In vitro* pharmacological screening relative to nicotine as a standard.
- *In vivo* pharmacological screening.
- 28 day repeat dose toxicity in mouse
- Developmental toxicity screening study.

Pharmacology

- Isolated guinea-pig ileum.
- Isolated rat phrenic nerve diaphragm preparation.
- Isolated chick biventer cervicis preparation.
- Irwin screen in the mouse.
- Motor co-ordination in the mouse (rota-rod)

Toxicology

- Sub-acute study in mice dosed by gavage once a day for 28 days at 0, 0.12, 0.6 or 3 mg/kg bw anatoxin a HCl (0.098, 0.49 and 2.49 mg/kg anatoxin a). Same examinations as for microcystin.
- One group of time mated female mice were dosed with either vehicle or 3 mg/kg anatoxin a HCl on days 6-15 of pregnancy and humanely killed on day 18. Developmental parameters examined as previously.

Pharmacology Results *In vitro*

- Results showed that anatoxin a is respectively 7, 136 and 24 fold more potent than nicotine in the three *in vitro* screens.

Pharmacology Results – *In vivo*

- Irwin screen – all dosed with 100 µg/kg died within 1 minute with symptoms of cholinergic stimulation and CNS effects. 2 died at 30 µg/kg. Others showed increased respiration, salivation and hyperactivity with recovery within 2 mins but no effects on screen parameters.
- Rota-rod deaths in all dosed groups but others on recovery no effect on rota-rod parameters

Sub-acute Toxicity Results

- 3 deaths, one male at 0.6 mg/kg and one female receiving 3 mg/kg. Both clinically unremarkable as was necropsy and histopathology failed to reveal a cause of death.
- Nothing in food intake, bodyweights or histopathology. Minor diffs in haematology and biochemistry in absence of other changes considered to be not of biological significance.
- NOAEL 0.098 mg/kg bw anatoxin a. The true NOAEL may be 2.46 mg/kg bw anatoxin a, but not able to determine cause of death for the two that died.

Developmental Toxicity Results

- No premature maternal deaths or treatment-related clinical observations.
- No adverse effect on maternal bw or weight gain.
- No abnormalities observed at necropsy. Pregnancy incidence, mean implantation numbers, live fetuses, post-implanation losses, unaffected.
- No effect on fetal weight and no treatment related major fetal abnormalities.
- NOAEL - 2.46 mg/kg bw/day pure anatoxin a.

Conclusions Anatoxin a

- Potent nicotinic agonist that seems to act through effects on nicotinic acetylcholine receptors of adrenal medulla and sympathetic ganglia.
- If do not die, recovery complete.
- NOAEL in sub-acute tox study 0.098 mg/kg pure anatoxin a but true NOAEL may be actually be 2.46 mg/kg.
- Developmental NOAEL 2.46 mg/kg.

Final Comments

- 1 µg/litre is a reasonably conservative guideline for each; for total microcystins very conservative.
- Remember not all water drunk at one go and these are gavage studies. Continuous blooms are the biggest problem in developing countries.
- Provides a good benchmark for designing treatment.