COUMARIN DERIVATIVES

Definition [adapted from Lewis' Dictionary of Toxicology 1998]

Coumarin: A very toxic white crystalline substance ($C_9H_6O_2$) with an odour or new-mown hay, occurring naturally in many plants, especially in the seed or seed coat (testa) where it inhibits germination until removed or destroyed e.g. by photolysis.

Reference:

Lake BG (1999) Coumarin metabolism, toxicity and carcinogenicity: relevance for human risk assessment. *Fd. Chem. Toxicol.* **37**:423-453.

Murray RDH, Méndez J, Brown SA (1982) *The Natural Coumarins. Occurrence, Chemistry and Biochemistry.* John Wiley & Sons Ltd., Chichester.

☑ *Dihydroxycoumarin (dicoumarol)*

Core data

Syndrome names: sweet clover poisoning
Common sources:
mouldy hays/silage of

- *Melilotus* spp. (sweet clovers)
- *Anthoxanthum odoratum* (sweet vernal grass)

Animals affected: cattle, sheep, horses, deer
Mode of action: Fungi convert coumarol in the plants to dicoumarol, an analogue of vitamin K, thus interfering with synthesis of coagulation factors VII, IX, X & prothrombin
Poisoning circumstances: feeding mouldy hays or silage of coumarol-containing plants
Main effects: widespread haemorrhage
Diagnosis: dicoumarol assay (serum, feed)
Therapy: vitamin K_1 (transfusion if anaemia profound)
Prevention: avoid conditions leading to mould growth when harvesting source plants

Syndrome name(s):

- sweet clover poisoning
- ferulosis

Plant sources:

- mouldy hays/silage of ***Melilotus*** spp. (sweet clovers) [Family Fabaceae]
  - *Melilotus alba* (white sweet clover) – major source of dihydroxycoumarin toxicity of cattle in North America; occurs in Australia, but only 1 case is on record in Australia (Wignall *et al.* 1961).
  - *Melilotus indica* (Hexam scent) - occurs in Australia, but no cases of dihydroxycoumarin poisoning attributed to it are on record here.
- mouldy hay or silage of ***Anthoxanthum odoratum*** (sweet vernal grass) [Family Poaceae] [Davies & Ashton 1964, Cranwell 1983, Pritchard *et al.* 1983, Bartol *et al.* 2000, Garbor *et al.* 2001, Runciman *et al.* 2002]
  - one case in Victoria in cattle fed baled silage mouldy at its edges and comprising 90% *A. odoratum* ; 2 samples from the outer layer of silage contained 21 and 51 mg dicoumarol/kg while a core sample contained <1 mg/kg; serum dicoumarol was detected but not quantified in 2 cattle (Runciman *et al.* 2002)
  - one case in Tasmania in dairy calves of cattle fed hay comprising ryegrass and *A. odoratum* and moist when cut and baled; 27 & 39 mg dicoumarol/kg hay, serum dicoumarol in calf 3.1 mg/L, cow 14 mg/L (Garbor *et al.* 2001); a neighbour's herd was subsequently affected (Craig Dwyer, personal communication 2001).
  - coumarin acts as an allelopathic chemical providing *A. odoratum* with competitive advantage against other plants through secretion from roots (Yamamoto & Fujii 1997)
- *Ferula communis* (giant fennel) [Family Apiaceae] – occurs in southern Europe, North Africa, western Asia

Major occurrence: **Mouldy sweet clover poisoning** - North America (1 case seen in Australia [Wignall *et al.* 1961])

Fungi convert coumarol in sweet clovers or sweet vernal grass → dicoumarol, an analogue of vitamin K → interferes with synthesis of coagulation factors VII, IX, X & prothrombin → haemorrhage [cf. anticoagulant rodenticide poisoning]

Ferula communis is toxic in its normal state (fungal infection is not involved).

Toxicity:

Cattle, sheep, horses, deer susceptible

Cattle fed mouldy *Melilotus* spp. (Puschner *et al.* 1998)

- feeds with < 20 µg dicoumarol /g is apparently safe
- feeds with 20-30 µg dicoumarol /g potentially toxic if fed for a sufficient period (130 days)
- feeds with > 50 µg dicoumarol /g may cause severe signs within 15 days

Sheep fed *Ferula communis* @ 2.5 g/kg/day or more developed signs of poisoning (Shlosberg & Egyed 1985)

Clinical signs

- ± sudden death without signs of illness (Runciman *et al.* 2002)
- lethargy
- lameness, stiffness, reluctance to move
- subcutaneous haemorrhage → swellings from haematomas
- swollen joints (intraarticular or periarticular haemorrhage) - shoulders, carpus, tarsus (Runciman *et al.* 2002)
- recumbency
- mucosal pallor
- mucosal haemorrhages
- ± haematuria
- ± dysentery
- ± epistaxis
- ± vaginal bleeding
- ± bloat
- ± abortion
- ± haemolactia (blood in the milk) (Runciman *et al.* 2002)

Pathology

- ↑↑ prothrombin time, APTT & clotting time
- platelet numbers are normal
- anaemia
- extensive haemorrhages & haematomas

Diagnosis: clinical pathology; serum & feed dicoumarol assay

Therapy:

- as for anticoagulant rodenticide poisoning (above); vitamin K_3 is ineffective for prevention (Casper *et al.* 1989)
- adult cattle (Bartol *et al.* 2000): blood transfusion (2-6 L whole blood) especially when PCV <20% + vitamin K_1 @ 1-3 mg/kg IM (using the smallest practical needle and several injection sites; IV may cause anaphylaxis) every 12 hr for 5 days or until PT and APTT return to normal

References: Se308, Se146

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Daphnoretin (presumed toxin in Wikstroemia indica*)*

Wikstroemia indica (tie bush) [Family Thymeleaceae] is suspected of producing a haemorrhagic syndrome in farmed deer in Queensland on one occasion. Widespread haemorrhage occurred, but circulating platelet and leucocyte numbers remained normal [B.D.Hill unpublished data 1984, Dowling 1985, Se146, DM133]. Daphnoretin has been isolated from the plant stems (Kato *et al.* 1979).

Reference:

Dowling RM (1985) Tiebush: an old problem for a new industry. *Qd. Agric. J.* **111**(2): no pagination – printed on both sides of the rear cover.
Kato A, Hashimoto Y, Kidokoro M (1979) *J. Nat. Prod.* **49**:159 [cited by Murray *et al.* 1982 as reference 1623]

☑ *Furanocoumarins (furocoumarins, psoralens)*

Core data

Syndrome names:

- primary photosensitisation
- blue-eye

Common sources:

- *Ammi majus* (bishop's weed)
- *Apium graveolens* (celery) ± infected with microbes
- *Petroselinum crispum* (parsley)
- *Pastinaca sativa* (parsnip) ± infected with fungus

Animals affected: ruminants, horses, pigs

Mode of action: Form photo-adducts with nucleic acid pyrimidine bases and other molecules; **no** liver damage involved

Poisoning circumstances:

- ingestion or skin contact
- pigs: skin (snout, forelimbs) contact with vegetable waste including celery, parsley or parsnips

Main effects:

- corneal oedema with subsequent keratitis
- photosensitisation (without liver damage)
- vesication of snouts of white pigs

Diagnosis: syndrome + plant access

Therapy: general therapy for photosensitisation

Prevention: deny access

Syndrome:

primary photosensitisation
blue-eye
phytophotodermatitis (humans)

Chemical structure:

Major photoactive furanocoumarins in descending order of activity include psoralen, xanthotoxin (8-methoxypsoralen) and bergapten (5-methoxypsoralen) (Gray & Waterman 1978)

Plant sources in Australia:

Family Apiaceae (Umbelliferae):

Ammi majus (bishop's weed); whole plant & seeds toxic [Se8, Lopez *et al.* 1997, Dollahite *et al.*1978, Egyed *et al.* 1977, Trenchi 1960]

A case of primary photosensitisation in horses associated with *Ammi majus* was seen on the Darling Downs in 1990 in which 20 horses had a sudden onset of corneal oedema producing blindness and dermatitis of the white areas of the face and legs. Clinical chemistry indicated no liver dysfunction in 5 of the horses which were sampled. The herd was removed from the paddock concerned, treated with anti-inflammatory drugs and topical eye ointment and all but one horse recovered within 3 weeks. RA McKenzie, KF Sullivan & DR Cook, unpublished data 1991 cited in McKenzie (1994)

Apium graveolens (celery) ± infected with microbes (the fungus *Sclerotinia sclerotiorum* or the bacterium *Erwinia carotivora*) (Scheel *et al.* 1963, Beier & Oertli 1983); cases in pigs require differentiation from vesicular viral infections (Montgomery & Oliver 1985, Montgomery *et al.* 1987a,b)

Petroselinum crispum (parsley) (Griffiths & Douglas 2000)

Pastinaca sativa (parsnip) ± infected with fungus (*Ceratocystis* sp.)

Anethum graveolens (dill) – rare cases in humans (Mitchell & Rook 1979)

Family Fabaceae (Leguminosae):

Cullen patens [=*Psoralea patens*] (native verbine, Bullamon lucerne, spreading scurf-pea)

Cullen patens has been associated with primary photosensitisation of horses in the Warrego district of south-western Queensland. Affected horses have grazed lush stands of the plants and have developed corneal oedema, blepharospasm and oedema of eyelids, but no evidence of liver damage was detected with clinical chemistry. Horses recover quickly after access to the plant is denied. An experiment with one horse fed freshly collected *C. patens* produced blepharospasm and conjunctivitis after the horse ate about 6 kg of plant over 5 days. RA McKenzie, D Rossi & FJ Keenan, unpublished data 1991 cited in McKenzie (1994), RA McKenzie, unpublished data (2001) .

Family Liliaceae:

Cooperia pedunculata (thunder lily, giant prairie lily) ; see below; negative feeding trials in sheep and cattle have been done with material from Theodore (W.T.K. Hall, unpublished data, DPI Poisonous Plants Files 1972)

Family Rutaceae:

Citrus spp. (Gray & Waterman 1978, Stanley & Jurd 1971) including *C. aurantiifolia* (lime) (Nigg *et al.* 1993), *C. bergamia* (bergamot) (Kawaii *et al.* 1999)

Family Moraceae:

Ficus spp. (figs)

Primary photosensitisation was seen in 2 Quarter horses at Gatton in south-eastern Queensland in September 1992. The horses had eaten a large number of the leaves which had fallen from a large *Ficus macrophylla* (Moreton Bay fig) tree which was under heavy attack by fig psyllids (*Mycopsylla ficci* Tryon) and sooty mould (a saprophytic fungus using insect excreta as a nutrient source) and had lost nearly all its leaves. The horses had photophobia and corneal oedema but could still see enough to avoid obstacles. One of them had skin erythema of the unpigmented skin of the nose and 2 pasterns. Liver function tests indicated no abnormality. Both horses recovered after about 4 weeks. RA McKenzie & MC Campbell, unpublished data 1992, cited in McKenzie (1994).

Additional plant sources associated with toxicity incidents [North America, Europe]:

Family Apiaceae (Umbelliferae):

Ammi visnaga (bishop's weed, visnaga) [photosensitising capacity disputed by Egyed *et al* (1977)]

Cymopterus longipes (wild parsley) – poultry (Egyed *et al.* 1977, Shlosberg & Egyed 1977)

Cymopterus watsonii (spring parsley) – sheep (Binns *et al.* 1964), poultry (van Kampen *et al.* 1969, Williams & Binns 1968)

Heracleum mantegazzianum (cow parsnip, giant hogweed, cartwheel flower plant)

Family Liliaceae:

Cooperia pedunculata (thunder lily, giant prairie lily); both green and dead leaf material is toxic (Rowe *et al.* 1987, Casteel *et al.* 1988)

Family Rutaceae:

Thamnosma texana (Dutchman's breeches, Texas desert rue, blisterweed) (Oertli *et al.* 1983, 1984)

Family Moraceae:

Ficus spp.

Toxicity:

Fungal or bacterial infection of parsley or celery plants → ↑ furanocoumarin concentration as **phytoalexins**.

Phytoalexins are low-molecular weight antimicrobial chemical compounds synthesised by and accumulated in plants after exposure to micro-organisms (Paxton 1981).

Organ systems affected: eyes, skin

Mode of action:

Form photo-adducts with nucleic acid pyrimidine bases and other molecules - molecular oxygen not required for reaction

Conditions of poisoning:

- ingestion of pasture weeds or native pasture plant species containing furanocoumarins
- possibly ingestion of furanocoumarin-containing plants under stress, e.g. from insect attack , which boosts their furanocoumarin content (see *Ficus* sp.)
- **contact with green vegetable waste containing parsley, parsnips, celery** or combination of them and subsequent sunlight exposure; cases in grocery workers handling disease-resistant strains of celery (Berkley *et al.* 1986)

Clinical signs & Pathology:

Corneal oedema/keratoconjunctivitis is a feature of furanocoumarin photosensitisation (uncommon in other kinds); mydriasis & pigmentary retinopathy (black spots on the fundus of the eye) were seen in ducks fed *Ammi majus* seed (Egyed *et al.* 1977)

Skin vesication or bulla formation in **white pigs** (snout, forelegs) (Griffiths & Douglas 2000)

Diagnosis:

skin lesions of photosensitisation (± corneal oedema) + absence of liver damage determined by clinical examination for jaundice and confirmed by clinical chemistry (liver function tests on serum or plasma).

differentiate pig snout vesication from viral vesicular diseases of pigs (foot & Mouth disease, swine vesicular disease, vesicular exanthema, vesicular stomatitis); viral infections should produce fever and lesions on hind as well as fore feet and snout (Geering *et al.* 1995)

differentiate horse corneal oedema from acute eye infections or recurrent uveitis (*Leptospira* infection).

Therapy: see general therapy for photosensitisation (*q.v.*)

Prevention & control: deny access

References:

Review literature:

Se8

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☑ Phyto-oestrogens

Core data

Syndrome name: clover disease
Common sources:
- isoflavones in *Trifolium subterraneum, T .pratense*
- coumestans in *Medicago* spp.

Animals affected: sheep
Mode of action: mimic effects of oestrogen overdose
Poisoning circumstances: sheep grazing clovers
Main effects:
- ewes – cystic endometrial hyperplasia; permanent infertility
- wethers – enlarged teats, blocked urethra → bladder rupture

Diagnosis: syndrome + access
Therapy: nil
Prevention:
- low oestrogenic cultivars introduced have → mild infertility only
- strategic grazing (young breeders on lowest oestrogen content pastures)

Syndrome names: **`clover disease'**
Sources:
Family Fabaceae

Trifolium subterraneum (subterranean clover)
Trifolium pratense (red clover)
Medicago sativa (lucerne, alfalfa)
Medicago truncatula (barrel medic)
Stylosanthes humilis (Townsville lucerne) – insignificant amounts (Little 1969)

Toxicity:
- isoflavones in *Trifolium* spp.
  - very weakly oestrogenic, require large intakes for effects; >0.3% isoflavones → toxicity
  - P deficient soils enhance isoflavone content of clovers
- coumestans in *Medicago* spp.
  - fungal infection promotes coumestans as phytoalexins in *Medicago* spp./ damaged or stressed plants have greater concentrations
  - produced in response to irrigation with sewage water containing oestrogens (Shore 1999)

Mode of action: mimic effects of oestrogen overdose

Conditions of poisoning:
- ewes affected on clovers → **permanent infertility**
- wethers affected on clovers → **bladder rupture**
- cattle affected on fungal-infected lucerne → ± transient hyperoestrogenism, ± cystic ovaries
- cattle grazing lucerne irrigated with sewage water (Israel) (Shore 1999)

Clinical signs & Pathology:
- classical clover disease (**ewes**)
  - uterine prolapse
  - dystocia (uterine inertia or failure of cervical or vaginal dilation – Adams & Nairn 1983)
  - hydrops uteri
  - **cystic hyperplasia of endometrium**
  - permanent `defeminisation' after 1-2 years high intake → reduction in uterine size, metaplasia of mucosa of cervix → **↓ flock fertility**
- classical clover disease (wethers)
  - enlarged teats
  - bulbo-urethral gland cysts
  - blockage of urethra → **bladder rupture**

Diagnosis: syndrome + access

Therapy: nil

Prevention & control:
- **low oestrogenic strains of clover** introduced have ↓ occurrence of outright clover disease to mildly reduced fertility only
- adequate fertilisation of pastures
- graze prime young breeding stock on the least oestrogenic pastures (**strategic grazing**)
- genetic selection of more resistant sheep
- immunisation techniques have not prevented the disease
- haymaking may reduce the concentration of phyto-oestrogens; ensiling does not (Wilkinson 1999)

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Se87
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