Quantifying Evident Toxicity for the Fixed Dose Procedure

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Objectives

- Review the conduct of the fixed dose procedure (FDP) for assessing acute oral toxicity
- Describe the data currently collected
- Discuss how the data can be interpreted to identify evident toxicity

Presentation Outline

- Background and principles of the Fixed Dose Procedure
- Drivers for use of the FDP
- How the FDP is conducted
- Overview of the data collected
- Discussion of ‘Evident Toxicity’
- Concluding Comments

FDP - Historical background (1)

- **1984** BTS proposal for a new approach to classification on the basis of acute toxicity - introduced the concept of use of fixed doses and evident toxicity

- **1987** Publication of results of UK Validation Study which tested the concept and refined the method – 5 laboratories, 41 materials

- **1990** Publication of Results of large international validation study - 33 laboratories, in 11 OECD countries, 20 test substances

FDP - Historical background (2)

- **1992** FDP adopted by OECD as TG 420

  (2000 OECD GD 19 on humane endpoints published)

- **2001** Revised OECD 420 adopted

  (2001 TG 401 deleted)

FDP – Statistical Assessments

- **1992** Whitehead and Curnow “Statistical Evaluation of the Fixed Dose Procedure”
- **1995** Stallard and Whitehead “Reducing Animal Numbers in the FDP”
- **2004** Stallard and Whitehead “A Statistical Evaluation of the Fixed Dose Procedure”

International Validation Study - Conclusions

The FDP:

- Produces consistent results - not substantially affected by inter- laboratory variations
- Provides information on time to onset, duration and outcome of signs of toxicity as required for risk assessment
- Permits use of fewer animals than e.g. OECD 401
- Subjects animals to less pain and distress & less substance related mortality than e.g. OECD 401
- Provides results that allow classification (to the EEC system) broadly compatible with ranking based on LD50 values

Principles of the Fixed Dose Procedure

- Assessment of acute oral toxicity is based upon the observation of “evident” toxicity at one of four fixed dose levels (5, 50, 300 and 2000 mg/kg body weight)
- Only moderately toxic doses are administered
- Lethality or moribund status is not used as an endpoint
- Results can be used to classify the test substance, e.g. in accordance with GHS

Drivers for use of the FDP

Article 7 of 86/609/EEC ('Animal Welfare Directive')

- In a choice between experiments those which…….. cause the least pain, suffering distress or lasting harm and which are most likely to provide satisfactory results shall be selected

- All experiments shall be designed to avoid distress and unecessary pain and suffering to the experimental animals

Drivers for use of the FDP

UK Animals (Scientific Procedures) Act 1986

In order to grant a Project Licence, the Secretary of State must be satisfied that full use will be made of reduction and refinement strategies to minimise suffering by using

- the minimum number of animals

- protocols and endpoints which cause the least pain, suffering, distress or lasting harm (and which are likely to produce satisfactory scientific results)

Drivers for use of the FDP

Standard Condition of UK Project Licences

Appendix D, Item 6 of A(SP)A

- For any procedure, the degree of severity imposed shall be the minimum consistent with the attainment of the objectives of the procedure and this shall not exceed the severity limit attached to the procedure

A Brief Word on Severity Limits

- A Project Licence authorises performance of regulated procedures in the pursuit of defined objectives and sets severity limits for individual protocols

- Four categories

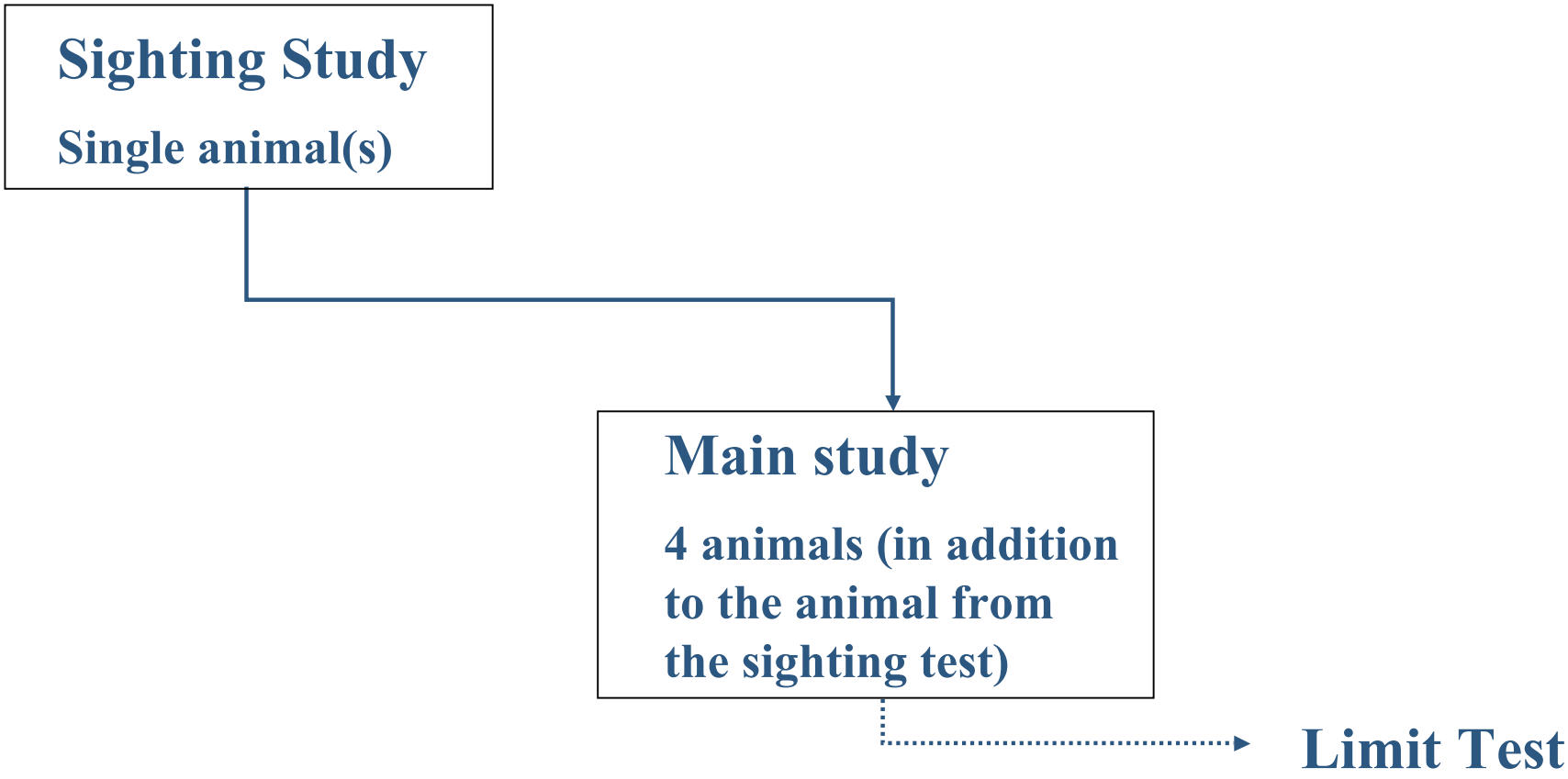
- The ATC & UPD can be used in the UK when scientifically justified, but the FDP is considered to be the most humane and so is the preferred method

OECD Guidance Document 19

Guidance Document on the Recognition, Assessment, and use of Clinical Signs as Humane Endpoints for Experimental Animals used in Safety Evaluation (2000)

- Guidance for determining when an animal is in a moribund condition or experiencing significant pain and distress
- Severe pain, suffering or death are to be avoided as endpoints

How the FDP is conducted: Overview



Each phase is conducted in a sequential manner according to the flow charts given in Annexes 2 and 3 to the Test Guideline

Study Procedure

Sighting Study

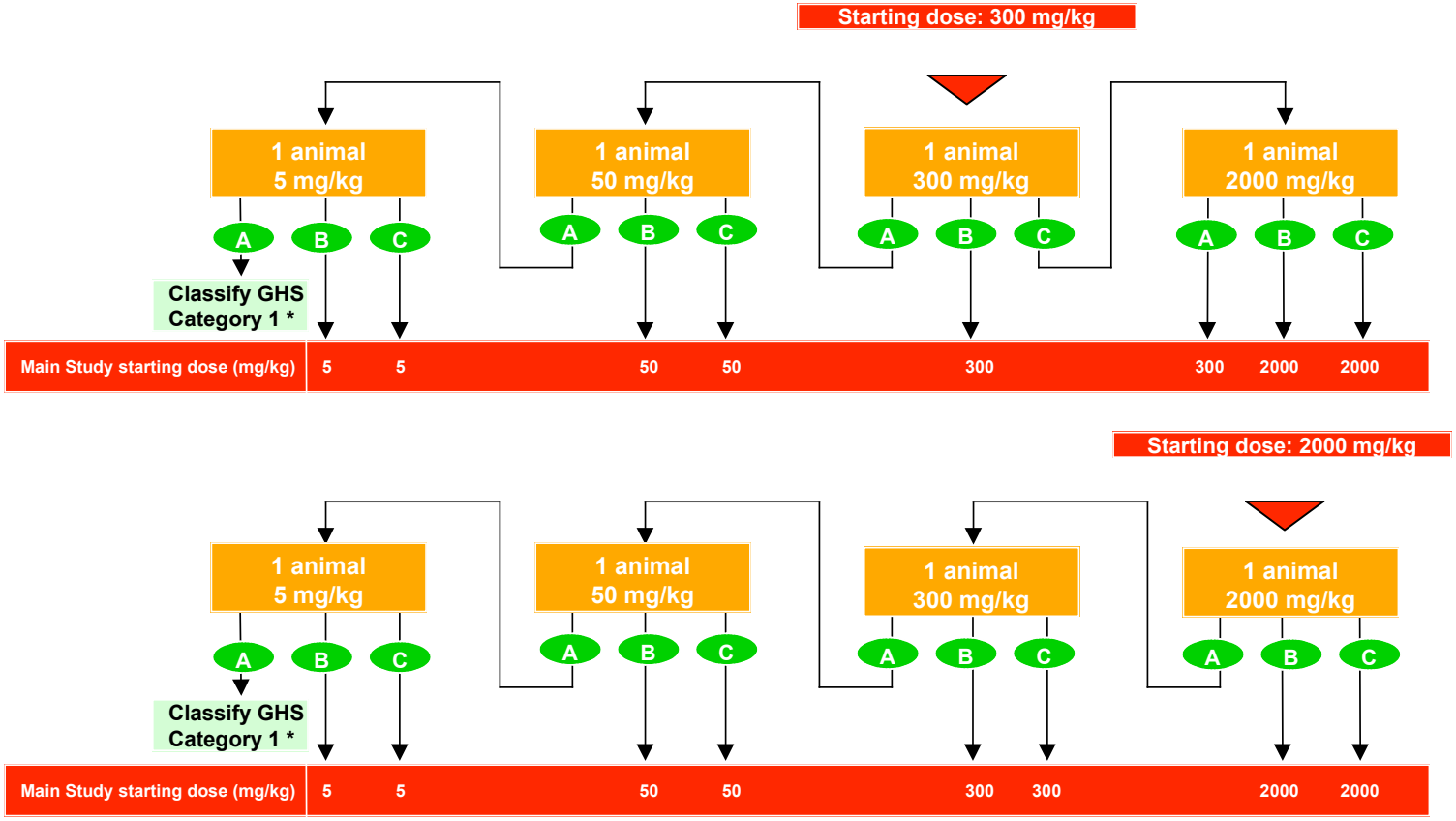
Choosing the Starting Dose

- A choice of four fixed dose levels: 5, 50, 300 or 2000 mg/kg bodyweight
- Taking all available information into consideration
- Objective: Choose a starting dose anticipated to cause evident toxicity; not mortality, severe signs of toxicity, corrosion/severe irritation, or marked pain and distress
- If there are no data available on the substance to be tested, then the default starting dose level is 300 mg/kg body weight

The Sighting Study

- Test material administered to a single animal at the chosen starting dose and the animal is observed carefully (24 hours minimum)
- Next step determined by reference to the flow chart in Annex 2 of OECD 420
- This allows identification of the starting dose for the Main Study (the dose level causing evident toxicity)

Annex 2: Flow Chart for the Sighting Study



Outcome

- A Death
- B evident toxicity
- C No toxicity

* for outcome A at 5 mg/kg there is an optional supplementary procedure to confirm the GHS classification: see paragraph 20.

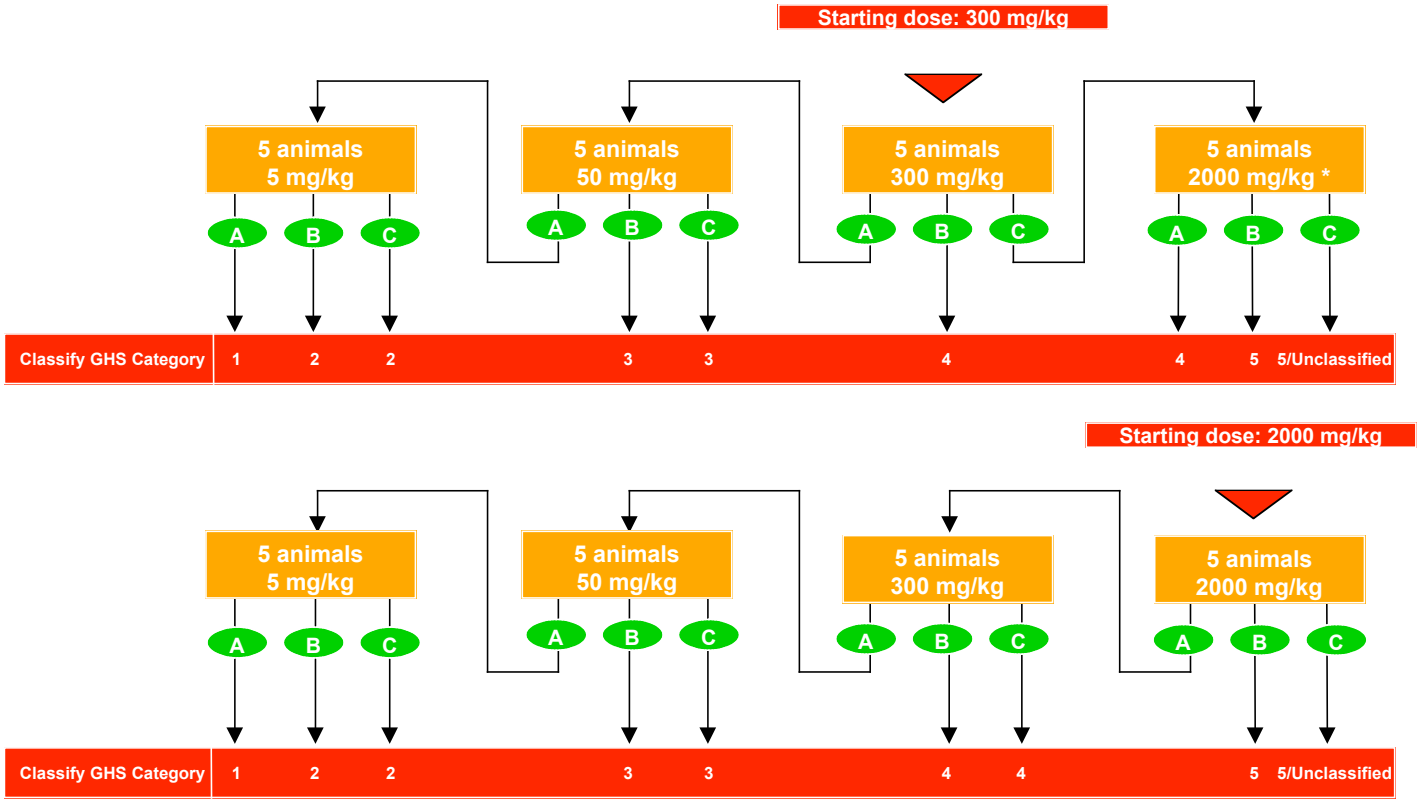
Study Procedure

Main Study

The Main Study

- Test material is administered to four additional animals at the fixed dose level that caused evident toxicity in the sighting test
- If no/mild toxicity was observed in the sighting test at 2000 mg/kg body weight, four animals are treated at this dose level (Limit Test)
- Animals are carefully observed for a minimum of 3 to 4 days and next step determined from the flow chart in Annex 3 Of OECD 420

Annex 3: Flow Chart for the Main Study



Outcome

A ≥ 2 Deaths

B ≥ 1 with evident toxicity and/or ≤ 1 death

C No toxicity

Group size

The 5 animals in each main study group will include any animal tested at that dose level in the sighting study

* **Animal welfare override**

If this dose level caused death in the sighting study, then no further animals will be tested. Go directly to outcome A

The Main Study

- This will allow classification of the test substance
- Do not re-visit a dose level that caused death in the sighting study
- In exceptional cases where testing at 5000 mg/kg body weight is required to satisfy a specific regulatory requirement, the procedure in Annex 4 of the test guideline must be followed

FDP: Data Collected

Observations	Procedure	Time Points
Clinical Observations	In accordance with test OECD 420, OECD Humane Endpoints Guidance Document No. 19 and Standard Operating Procedures	Once during first 30 minutes. Periodically during first 24 hours Daily thereafter Extend observation period beyond 14 days if necessary
Animal Body weights	In accordance with Standard Operating Procedures	Shortly before dosing. At least weekly thereafter At the end of the observation period
Pathology	Gross necropsy in accordance with Standard Operating Procedures Histopathology if considered appropriate	Necropsy all animals that: - die on test - are humanely sacrificed - are subject to a scheduled kill

Clinical Observations

- Cage-side observations & cautious interaction
- A list of commonly occuring signs can be useful i.e. a checklist
- Beware: Should not be regarded as complete
- Presence or absence of the clinical sign is recorded
- Severity recorded where appropriate
- Changes in external appearance, behaviour, activity, response to external stimuli
- Time of onset and duration of signs recorded

Body weights

- Individual body weights recorded
- More frequently than scheduled time points where effects suspected
- Continue to monitor body weight changes
- Be aware of reduced gains in body weight

Gross Pathology

- Record all gross abnormalities for major organs
- Note abnormal colouration, size, lesions, vascular effects, tissue adhesion, contents
- Preserve tissues for possible histology – rarely conducted in practice

Measurable Clinical Parameters

Not routinely included, neither are they requested for acute studies on chemicals e.g.

- Body temperature
- Heart rate
- Respiratory rate
- Clinical chemistry
- Haematology
- Food and water consumption

Assessment of neurotoxic effects

- Functional Observation battery not conducted
- No routine neuropathological investigations
- Specific signs may indicate possible neurotoxicity e.g. tremors, diarrhoea, salivation, convulsions, paralysis, motor incoordination, behavioural changes

How is evident toxicity identified?

- Currently, there are no globally agreed systems for quantifying evident toxicity
- Identification of evident toxicity is currently based on the nature, severity and duration of the clinical signs of toxicity, including body weight effects

How is evident toxicity identified?

- Exercise professional judgement
  - Experienced animal technicians
  - Study Director
  - Veterinarian
  - Named Animal Care and Welfare Officer (NACWO)
  - In conjunction with guidance documents (S.O.P's)

- Important pathological conditions are rarely seen without some signs of toxicity being displayed, so are less of a consideration

A perceived difficulty?

- ‘Severity’ of toxicity may be considered as a continuum

- Traditionally, classification systems are not based on the specific signs of toxicity or severity of toxicity, but upon the LD50

- Classification based on ‘evident’ toxicity might present a challenge this traditional approach

- Easier to identify in practice, than it is to define based on data

Can you see what it is yet?!!

ataxia

Severity

piloerection

pallor

Duration

tremor

lethargy

Reduced bodyweight gain

ptosis

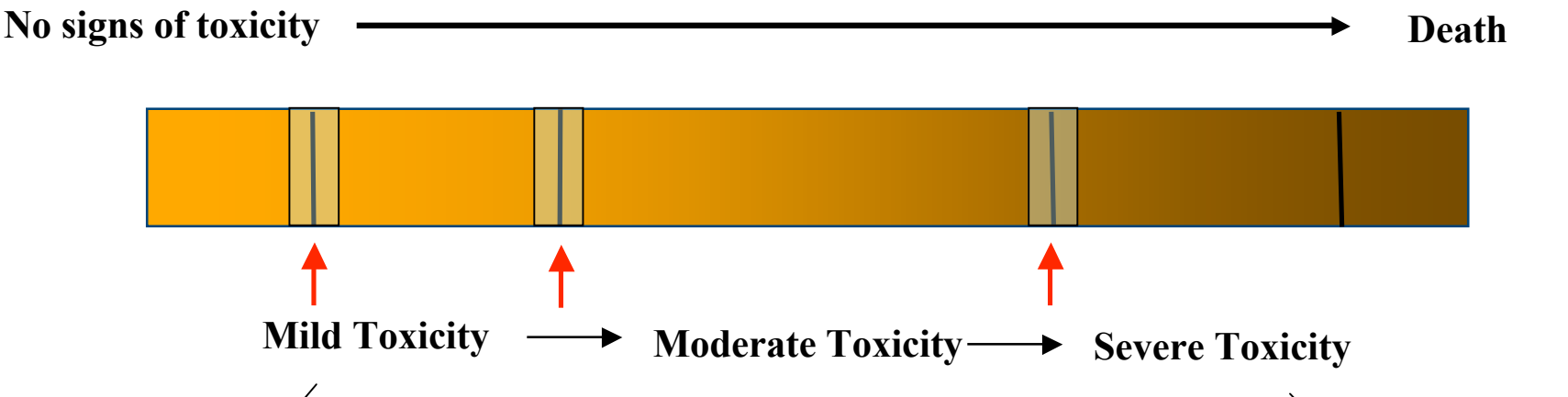
chromodacryorrhea

dyspnoea

The complete picture

Evident Toxicity

How is evident toxicity identified?



First detectable signs of a departure from normal appearance of well-being

Evident Toxicity

Clear signs of toxicity

Moderate toxicity

(not impending death or moribund condition)

Moribund status

- OECD GD 19
- Toth (2000)

How is evident toxicity identified?

- Production of a comprehensive list or set of conditions that can be reliably used to identify evident toxicity, or indeed any level of toxicity, pain or distress, based upon the clinical signs is likely to present a challenge
- Guidance on the identification of what conditions constitute evident toxicity may be feasible and would be useful
- Guidance on identification of clinical signs, such as OECD GD 19 and other publications, are useful but familiarity with the species and strain of animal is also of great importance

Guidance: BTS (1987)

- Minor effects such as diarrhoea, piloerection ungroomed appearance do not meet the criteria for evident toxicity
- A trained observer is unlikely to have difficulty in identifying animals showing marked signs of toxicity

Guidance: OECD Definition of Evident Toxicity

*"a general term describing **clear signs of toxicity** following the administration of test substance such that at the next highest fixed dose either severe pain and enduring signs of severe distress, moribund status, or probable mortality in most animals can be expected"*

Ref. Annex 1 of OECD TG 420

Guidance: Severity Limits under A(SP)A 1986

- **Mild Severity** - gives rise to slight or transitory minor adverse effects
- **Substantial Severity**– results in major departure from the animal's usual state of health or well-being
- **Moderate Severity** – Non-lethal toxicity tests

Guidance: Buckwell (1992)

Mild	Moderate	Substantial
Reduced weight gain	Weight loss of up to 20%	Weight loss greater than 25%
Food and water consumption 40–75% of normal for 72 h	Food and water consumption less than 40% of normal for 72 h	Food and water consumption less than 40% for 7 days, or anorexia (total inappetence) for 72 h
Partial piloerection	Staring coat—marked piloerection	Staring coat—marked piloerection—with other signs of dehydration such as skin tenting
Subdued but responsive, animal shows normal provoked patterns of behaviour	Subdued animal shows subdued behaviour patterns even when provoked.	Unresponsive to extraneous activity and provocation
Interacts with peers	Little peer interaction	
Hunched transiently especially after dosing	Hunched intermittently	Hunched persistently ('frozen')
Transient vocalization	Intermittent—vocalization when provoked	'Distressed'—vocalization unprovoked
Oculo-nasal discharge transient (typically signs of chromorhino-dacryorrhoea in rodents)	Oculo-nasal discharge persistent	Oculo-nasal discharge—persistent and copious
Normal respiration	Intermittent abnormal breathing pattern	Laboured respiration
Transient tremors	Intermittent tremors	Persistent tremors
No convulsions	Intermittent convulsions	Persistent convulsions
No prostration	Transient prostration (less than 1 h)	Prolonged prostration (more than 1 h)
No self-mutilation	No self-mutilation	Self-mutilation

Modified version from FELASA Report in Laboratory Animals (194) 28, 97-112

Brief Overview of a Data Set

- 438 FDP data sets, 2001 to date
- Various Product Types:

- Industrial chemicals e.g. raw materials, intermediates, catalysts etc.
- Pharmaceutical intermediates and raw materials
- Agrochemicals actives, formulations
- Petrochemicals e.g. base oils, fuels, additives
- Biocides
- Pigments, dyes, inks

Brief Overview of a Data Set

GHS Category	Acute toxicity range estimate (mg/kg)	Number of Studies	%
5	2000 ≤ LD50 ≥ 5000	357/438	82
4	300 ≤ LD50 ≥ 2000	71/438	16
3	50 ≤ LD50 ≥ 300	9/438	2
2	5 ≤ LD50 ≥ 50	1/438	<1
1	0 ≤ LD50 ≥ 5	0/438	0

Brief Overview of a Data Set

- Majority of products tested (82%) were GHS Category 5 (EU Unclassified)
- Prediction of low toxicity using non-animal methods would result in large savings in animal numbers
- Much data reviewed by peers, regulatory agencies with no known rejections or comments
- Indicates a general level of satisfaction with the data for classification purposes

Brief Overview of a Data Set

- Demonstrated that some GHS 5 materials may produce clinical signs of toxicity, or even an isolated death/humane kill, at the 2000 mg/kg dose level - others may produce no signs of toxicity
- In many studies - mild or no signs of toxicity are observed
- Evident toxicity is not always produced. Intermediate doses would be needed to induce evident toxicity

Brief Overview of a Data Set

- Some occasions, where no effects at one fixed dose level, may be deaths/humane kills at the next higher dose level (steep dose-response curve)
- Hunched posture, lethargy and piloerection (H,L,P) seen quite commonly and would not be regarded as evident toxicity unless no signs of recovery
- Clinical signs of evident toxicity have usually disappeared by end of the 14-day observation period. Persistent minor signs, should be considered to represent evident toxicity

Example of data - Limit Tests

Two Limit tests conducted with different chemicals at 2000 mg/kg body weight

Study 1: Hunched posture (H) up to 4 hours. Normal after 24 hours. Mild toxicity of short duration

Study 2: Hunched posture (H), respiratory effects (Rd), ataxia (A) after 30 minutes. Peak effects at 2 hours. Some effects up to 4 hours. Normal after 24 hours. Moderate toxicity, short duration (Evident toxicity)

Both GHS Category 5 Classification

Example of data – steep dose response curve

- Sighting test : 2000 mg/kg

No clinical signs on day of dosing but animal found dead on Day 1

- Sighting test : 300 mg/kg

No clinical signs of toxicity

- Main Study: 300 mg/kg

No clinical signs of toxicity

- GHS Category 4 Classification

Example of data – Two different chemicals

- For both chemicals the sighting test was conducted at 300 mg/kg. There were no clinical signs of toxicity

- For both chemicals the main study was conducted at 2000 mg/kg. Onset of clinical signs occurred at 30 to 60 minutes. And persisted at the 4-hour observation. The clinical signs were similar for both chemicals except that in one study occasional tremors were noted. All animals appeared normal within 24 to 48 hours

- Both chemicals exhibited evident toxicity and were classed as GHS Category 5. Did they have the same or different mechanisms of toxicity?

Concluding comments

What is ‘Evident Toxicity’ ?

It is:

- A non-lethal toxicity endpoint
- A description of clear signs of toxicity
- Equivalent to moderate toxicity

Concluding comments

What is ‘Evident Toxicity’

It is not:

- Mild toxicity
- Severe toxicity
- Impending death/ Moribund status

Concluding comments

- In the FDP, death, pain and suffering are minimised as evident toxicity is used as the endpoint
- Some guidance is available for identifying evident toxicity and additional guidance would be useful. However, a complete definition of evident toxicity may not be possible, or even necessary
- Evident toxicity might be desirable in studies designed to provide information on mechanisms of toxicity.
- Higher levels of toxicity might compromise the animals to such an extent that objective measurements may not be practical

Concluding comments

- Satellite animals could be included for conducting interim measurements or to ensure presence of some signs of toxicity
- Greater importance should probably be attached to the time of onset, nature, severity and reversibility of effects seen in acute regulatory acute toxicity studies for risk assesment
- After seven years use of the FDP for regulatory purposes there may be opportunities for review of existing data sets

Thank you for your attention

Questions ?