



Criteria and decision-making process for anticipating endpoints in preclinical development studies

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Why define warning criteria ?

Endpoint criteria are too late

- Based on OECD guidance

Anticipate a possible suffering or pain

- Implementation of the 3Rs roles
- Animal care takers and technician expectation

OECD Environmental Health and Safety Publications

Series on Testing and Assessment

No. 19

Guidance Document on the Recognition, Assessment, and Use of Clinical Signs
as Humane Endpoints for Experimental Animals
Used in Safety Evaluation



An experience

- **Review the process chronology**
 - ┌ Process
 - ┌ Implementation
- **Deliverables**
- **Establish the results**
 - ┌ pros and cons
- **Provide perspective**



Process: creation of a working group

The task force

- including all the actors of a toxicology study
- **Study Directors, Toxicologists**
- **Pathologists, Clinical pathologists**
 - ┌ Provide the feedbacks related to the diagnosis
- **Study Technicians, Animal Care-takers**
 - ┌ As the key actors
 - ┌ Emphasize their roles
- **Veterinarians**
 - ┌ From the Lab Animal Services & Welfare



Process: define objectives & deliverables

Two items

- The most appropriate and early endpoints for safety pharmacology studies and toxicology studies
- To help the study director to make the best and fastest decision in the course of these studies

This process lead to the development of two deliverable documents

- a Clinical Evaluation Chart
- a Decision-Making Chart



Implementation of a new policy on warning criteria

The Clinical Evaluation Chart

- **An original concept in animal welfare**
 - └ since it includes warning criteria that replace the classical endpoints
- **Facilitate identification of early relevant clinical signs**
 - └ Prevent significant adverse impact on the animal's welfare.
- **Lead to a temporary or permanent interruption of studies**
- **Applied to individual or groups of animals**

- **Posted in all animal rooms of the facility**
 - └ To allow everyone to initiate the process contained in the Decision-Making Chart



Clinical evaluation chart (1/3)

LESS SEVERE CLINICAL SIGNS <i>In the presence of at least three signs, call the Study Director</i>	MORE SEVERE CLINICAL SIGNS <i>In the presence of at least one sign, call the Study Director</i>
Respiration	
Respiration changes	Abnormal respiratory sounds Labored breathing Suffocation
General aspect	
Abnormal staining of the skin (other than injection or sampling sites) Pale mucosa Staining around muzzle (rodents) Generalized flaccidity Abdominal swelling Piloerection Hunched back Stained anogenital area	Persistence of skin pleat Yellow and/or blue coloration of mucosa Generalized muscular rigidity Cold to touch
Eyes	
Chromo-dacryorrhea Eyelids closed or half-closed and/or runny eyes	Injury of the eye and/or annexes







Clinical evaluation chart (2/3)

LESS SEVERE CLINICAL SIGNS <i>In the presence of at least three signs, call the Study Director</i>	MORE SEVERE CLINICAL SIGNS <i>In the presence of at least one sign, call the Study Director</i>
<p> Wound / Trauma</p>	
<p>Lesion (without any skin injury)</p> <p>Mass</p>	<p>Severe lesion (self-mutilation, running wound, ulcerated mass ...)</p> <p>Traumatism (fracture ...)</p> <p>Poor local tolerance (I.V. or S.C.): extended lesion and/or difficulty/impossibility to dose the animal</p>
<p> Behavior / Movements</p>	
<p>Agitation / Aggressiveness</p> <p>Abnormal vocalization</p> <p>Reduced motor activity</p> <p>Reduced reactivity</p> <p>Moderate incoordination</p> <p>Resistance during dosing and/or handling</p> <p>Circling displacements</p>	<p>Absence of motor activity</p> <p>Convulsions</p> <p>Lying on cage floor</p> <p>Difficulty / impossibility to move</p> <p>Marked incoordination (i.e. causing falls)</p> <p>Generalized tremors</p> <p>Convulsions</p>



Clinical evaluation chart (3/3)

LESS SEVERE CLINICAL SIGNS <i>In the presence of at least three signs, call the Study Director</i>	MORE SEVERE CLINICAL SIGNS <i>In the presence of at least one sign, call the Study Director</i>
 Excretion	
Liquid feces	Absence of feces (<i>rodent only</i>)
Mucoid feces	Red feces / Black feces (> 2 days)
Regurgitation / Emesis (<i>non rodents</i>)	Red urine
 Reproduction	
Genital discharge	Fetuses or fetal material in the cage
 Body weight evolution	
Body weight loss < 10%	Body weight loss > 10%
 Food consumption	
Food intake decreased or absent	Absence of food intake > 2 days



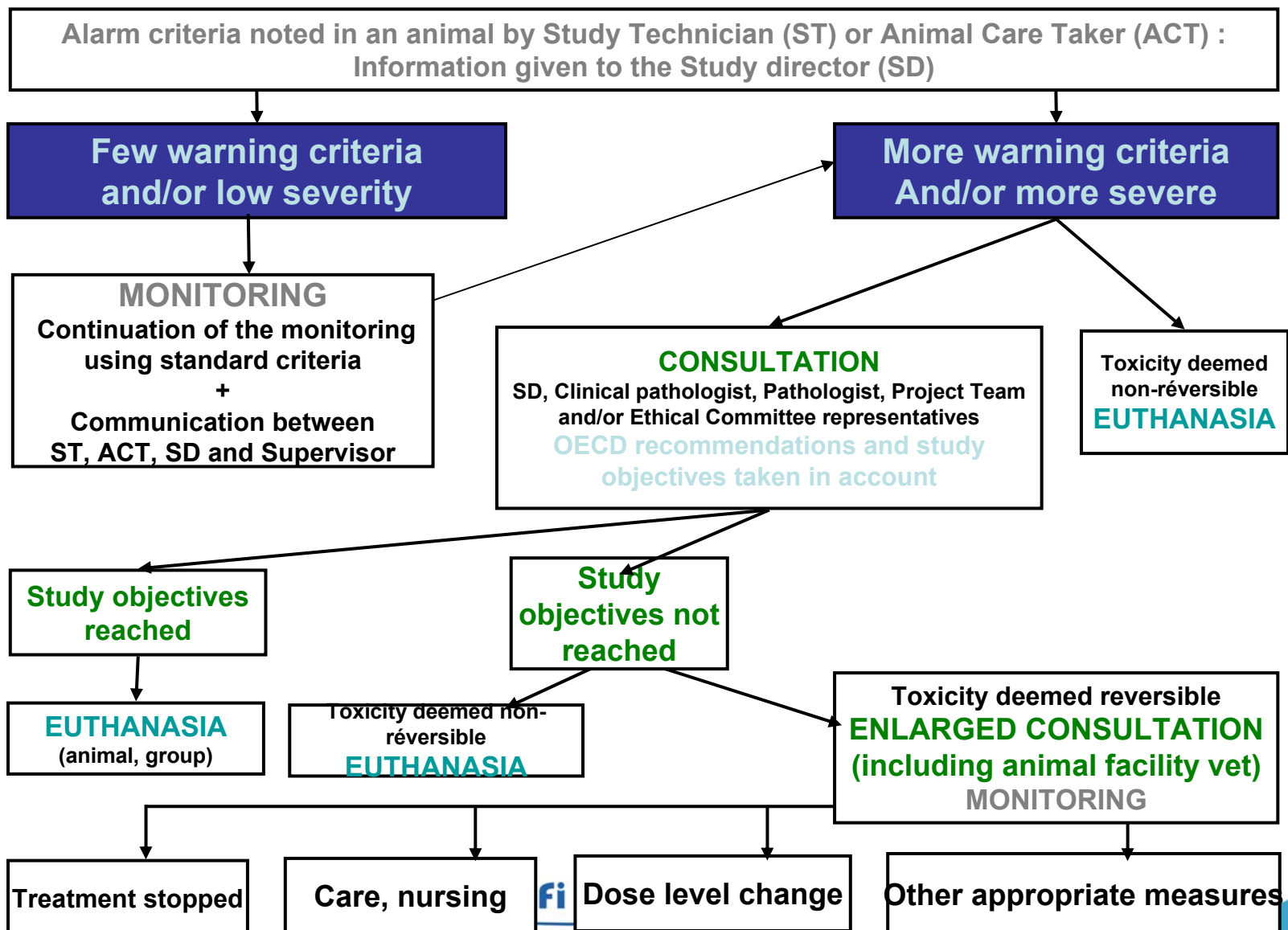
Implementation of a new policy on warning criteria

The Decision-Making Chart

- **Designed to help in the decision-making process**
- **Leading to a rapid intervention**
- **The result of this intervention depends on the clinical signs of the animal,**
 - 【 balanced against the objectives of the study
 - ▶ Without jeopardizing study objectives.
- **The Study Technician, the Animal Care-takers or anyone participating in the study**
 - 【 May initiate this process resulting in an overall improvement in animal welfare



Decision making chart





Results: examples (1/2)

Pilot dose range-finding study in rabbits

- **Treatment staggered for the different dose levels**
- **3 to 4-day period without food intake**
 - └ Lead to the interruption of treatment
 - └ Return to the colony for recovery
- **Relevance of the decision of termination**
 - └ Reached the study objectives
 - └ Validated at the next lower dose

2-week oral RF toxicity study in juvenile rats

- **Anticipated euthanasia of all the rats of highest dose**
 - └ due to body weight loss on Post natal Day 5



Results: examples (2/2)

Single dose 30-min IV in cynomolgus (anticancer)

- Decision of euthanasia on first clinical signs at the highest dose
- Confirmed by the clinical pathology analysis

Single dose 60-min IV in dogs (cytotoxic)

- Decision of euthanasia on clinical pathology analysis
 - ┌ Before severe clinical signs



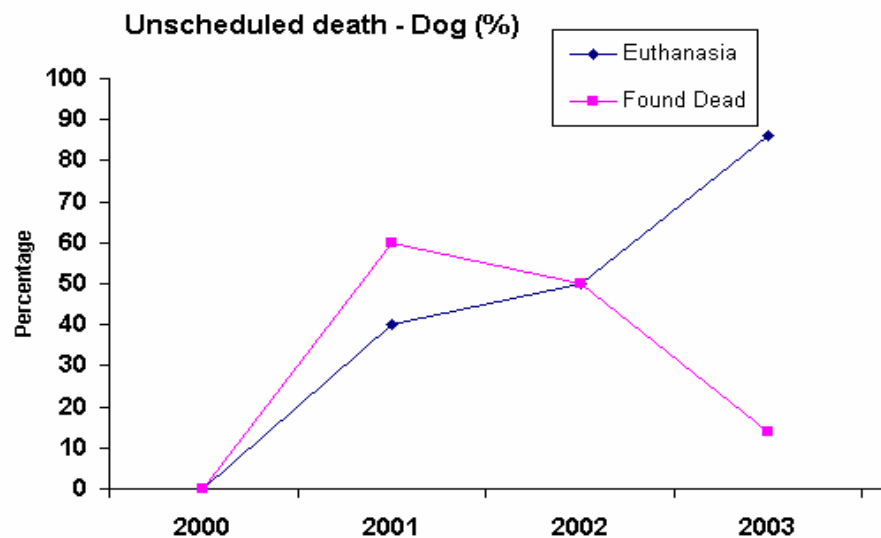
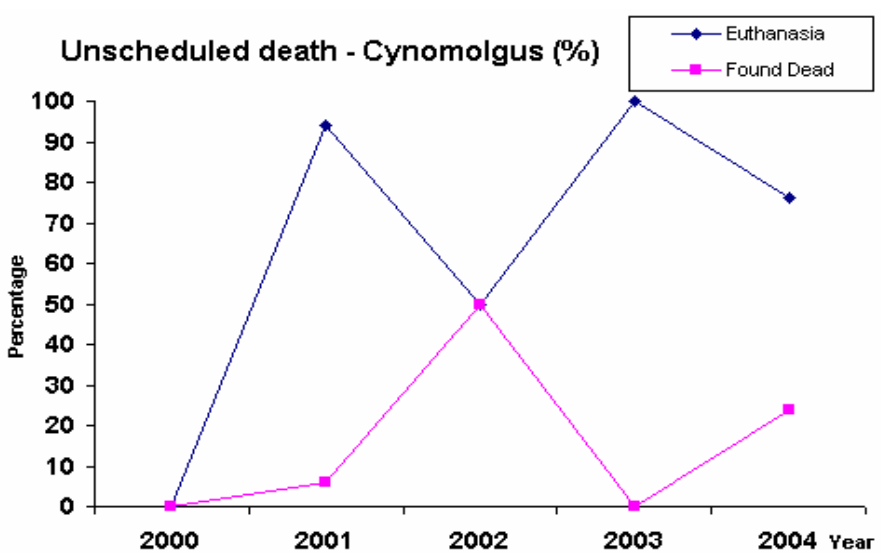
Results: analysis

Large increase of call to the scientists

- Involvement of the animal Caretakers and Study Technicians

Decrease of the number of « found dead »

- Number of anticipated euthanasia increased
- Comprehensive terminal examination
 - └ For clinical pathology and necropsy



Perspective and conclusion

Benefits

● High awareness by all the actors

- ┌ Animal care takers
- ┌ Technicians
- ┌ Clinical pathologists
- ┌ Pathologists
- ┌ Study directors

● Sense of urgency

- ┌ Earlier euthanasia



Consequences

● Changing the attitude of scientists

- ┌ Animal ethic taking into consideration by scientists

— This process reinforced a real culture of care within the group.