Assessing welfare and severity of GA mice under Directive 2010/63/EU

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- The Directive and the EU - Expert Working Group
- The Complexities of Assessing Welfare
- Moving Towards an Reporting Actual Severity

Genetically Altered Animals under Directive 2010/63/EU

Article 3

Procedure - *Includes* – **creation and maintenance of a GM animal** which may experience *pain, suffering, distress or lasting harm equivalent to, or higher than, that caused by the introduction of a needle.*

Article 17(1) states that

" A procedure shall be deemed to end when no further observations are to be made for that procedure or, ***as regards new genetically modified animal lines, when the progeny are no longer observed or expected to*** *experience PSDLH equivalent to, or higher than, that caused by the introduction of a needle."*

- **GA animals** = include genetically modified (transgenic, knock-out and other forms of genetic alteration) and naturally occurring or induced mutant animals as per the definition in Article 3(1).
- **Defining factor :** intended non-harmful or harmful phenotype
- An animal with a **harmful phenotype** is to be understood as an animal who is likely to experience, as a consequence of the genetic alteration pain, distress, suffering or lasting harm, higher than that caused by the introduction of a needle in accordance with good veterinary practice.

EWG on Severity Assessment relating to the Creation and maintenance of GA animals

Objective

- Develop a consensus document on general principles for the severity assessment of GA animals
- Publish this on the Commissions web-site to facilitate consistent interpretation and application of the Directive 2010/63/EU
- Develop this to inform Statistical Returns

Goal of Working Group

- Define criteria for “creation” and “establishment” of a GA line
- Define criteria to differentiate between a “harmful” from “non-harmful” phenotype
- Develop guidance on severity categorisation of “harmful phenotypes” and provide examples
- Develop guidance on the severity of sampling methodologies used to obtain tissue for genotyping

Why should it be so difficult?

A normal mouse?

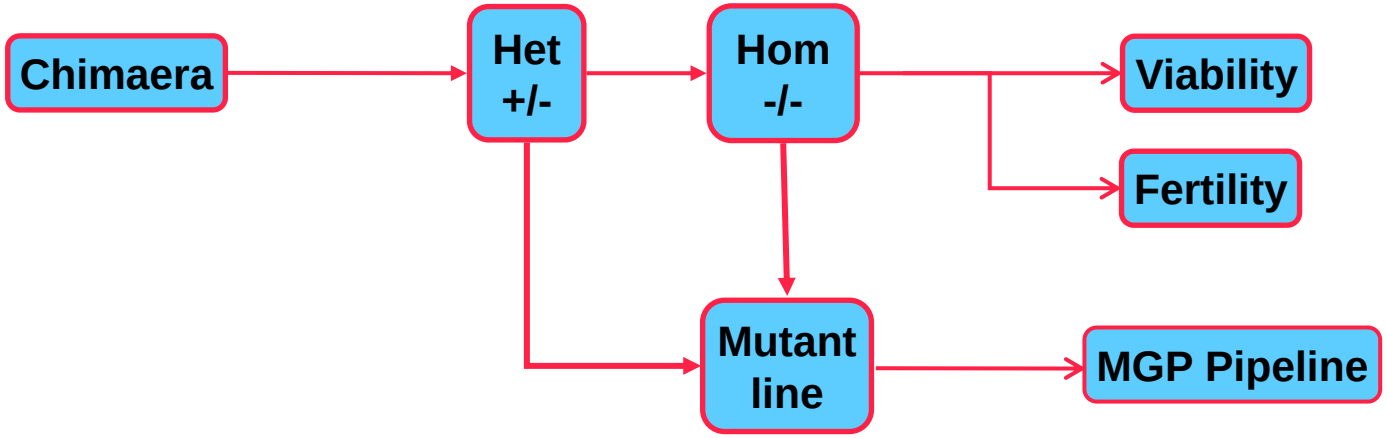
Healthy and thriving animals in their optimal healthy condition conforming to the breeding parameters and traits relating to their genetic background

- C57BL6/N 4.5 – 5.5 pups per litter

Multiple factors deemed harmful under directive 2010/63/EU may apply

- Genetic manipulation
- Breeding of harmful phenotypes
- Procedural burden
- Cumulative effect of the above

At what point will harmful phenotypes manifest – MGP Sanger



Genotype	Total # lines	# ≥1 hit	% ≥1 hit	Average hits/genotype
HOM	160	103	64%	3.9 (627/160)
HET	90	38*	42%	1.0 (93/90)

Haploinsufficiency relatively common

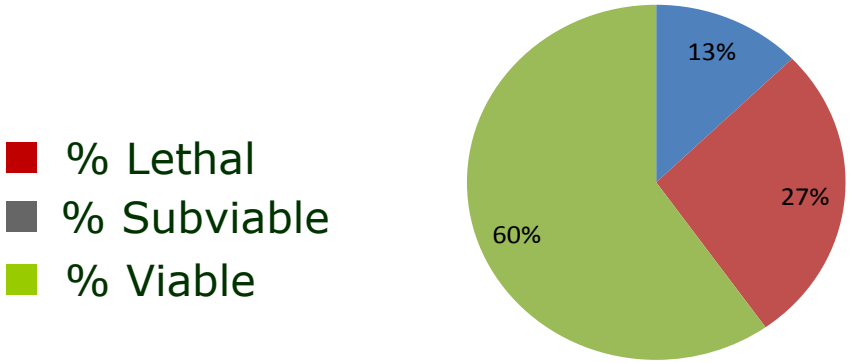
* Excluding viability

Homozygous viability

612 lines assessed by het inter-crossing

- P14, ≥ 28 progeny

Status at P14	Definition	Number of lines
Lethal	0% homs	168 (27%)*
Subviable	>0% and ≤13% homs	78 (13%)
Viable	>13% homs	366 (60%)



*Jackson Labs Database
1534/5166 (30%) lines on MGI reported with embryonic/ perinatal lethality

Understanding the diverse phenotypes of GA mice:

Genotypes Compared	Colony Prefix	Allele Name	Body Weight Curve (HFD)	Dysmorphology (56)	Hair Follicle Cycling	Indirect Calorimetry (7)	Core Temperature/ Stress induced	ip-GTT (2)	DEXA (9)	X-ray Imaging (41)	Heart Weight (3)	Open Field (13)	Grip Strength (7)	Modified SHIRPA (21)	Hot Plate (2)	Eye Morphology (34)	ABR (6)	Full Clinical Chemistry (26)	Peripheral Blood Lymphocytes (9)	Haematology (10)
wt v hom	MAHN	Mysm1<tm1a(KOMP)Wtsi>																		
wt v het	MASA	Ndufs3<tm1a(EUCOMM)Wtsi>																		
wt v hom/hemi	CRET	Hprt1<Tg(CMV-Cre)Brd>																		

Many models remain remarkably “normal” while some may express a phenotype when combined with the appropriate model to create a desired controlled alteration

Finding ways to differentiate between these models allows us to tailor our care and breeding strategies to their needs.

Welfare Assessment

Principal

Should include an assessment of general health, welfare and behaviour together with a review of production parameters such as breeding and growth performance which will ideally be compared with an appropriate non-GA background strain.

Outcome for a GA model

Lines may be considered "non-harmful" where there is no expected impairment of the well-being or general condition of the animals, and where production parameters do not significantly differ from relevant background non GA lines.

Creation and Establishment of a GA model

Creation - Relatively straight forward!

- Genetic manipulation e.g. ES cell and subsequent microinjection
- Breeding together of two GA lines
- Recipient female, vasectomised males
- Birth of potential mutants
- Genotyping and confirmation that mutation is present

- A new strain or line of genetically altered animals is considered to be "established"

  - When transmission of the **genetic alteration is stable,** which will be a **minimum of two generations**, and
  - Once an **initial welfare assessment** has been **completed**

Welfare Assessment

Should Include animals of representative age groups

- Soon after birth, around weaning and again following sexual maturity
- A minimum of 7 males and 7 females representative of the mutation sampled from more than one litter
- Data from a minimum of two breeding cycles (from F2 onwards)
- Comparisons made wherever possible with similar non GA animals.

Additional time points

- As considered appropriate by a prospective review of the potential impact of the gene alteration e.g. where there is an age dependent onset of disease

- In conjunction with the experimental design of your research

MOUSE WELFARE TERMS

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Mouse Welfare Terms

Welcome to a website dedicated to standardising the way we describe different characteristics of laboratory mice which may impact on their welfare. The team who have developed the listing of terms is lead by mouse care staff and have worked with veterinarian advice to compile a list of controlled language for cage-side descriptions of any mice that may cause concern while being used for scientific research.

The ultimate aim of this listing is to highlight welfare concerns associated with the maintenance of laboratory mice and any phenotypes that should be conveyed when either using these models locally or transferring to other facilities.

We hope that this list- which we have called Mouse Welfare Terms will be dynamic and that anyone wishing to be involved and would like to add or edit details within the list will contact the group.

CONTACT details: comments@mousewelfareterms.org

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Developed across two large production and Phenotyping facilities
Now adopted by their European partners

Eyes open - to timed observations

Parturition

P0:

- Milk spot present
- Eyes closed

Ear ID

P14:

- Teeth erupted
- Eyes open
- Activity when placed back into the litter
- Skin Pigmentation
- Skin Condition
- Skin Tenting
- Coat intact

Spns2^{tm1b} -/-

Weaning

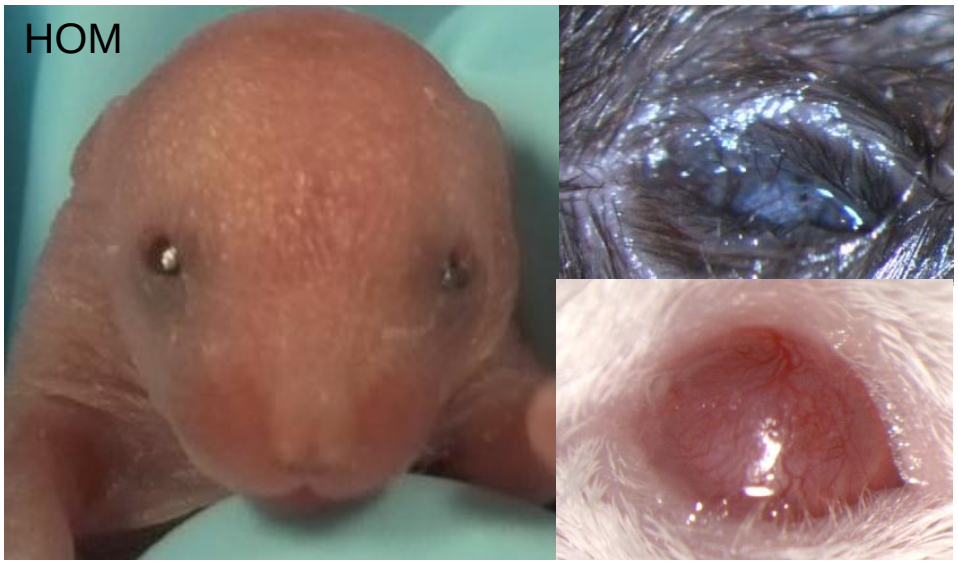
P21:

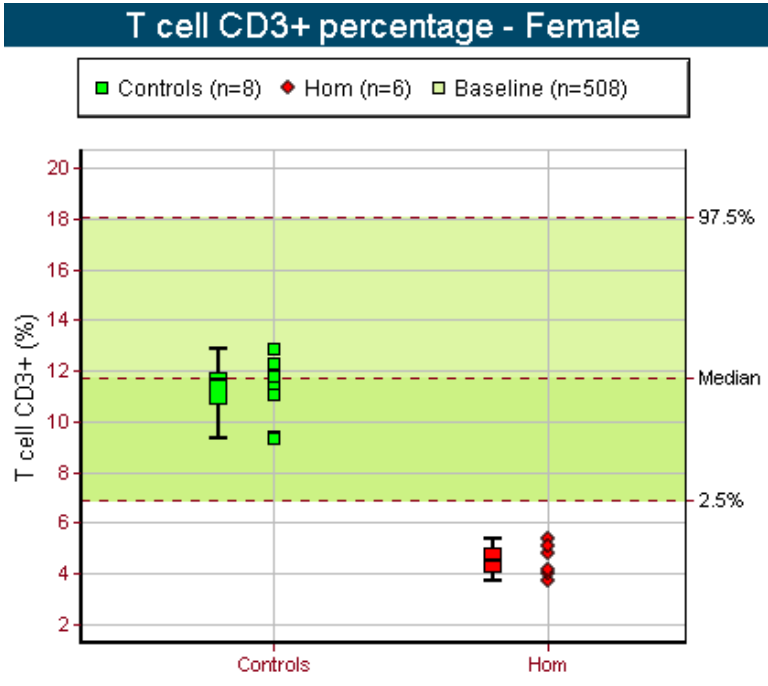
- Behaviour in cage
- Posture
- Runted
- Coat intact
- Coat colouration
- Coat texture
- Genitalia
- Genitalia morphology
- Head size
- Head morphology
- Ear size
- Ear morphology
- Eyes
- Eye morphology
- Incisor
- Incisor colour
- Mouth morphology
- Whiskers
- Limbs
- Gait
- Paws
- Digit Morphology
- Digit Count
- Nails
- Tail

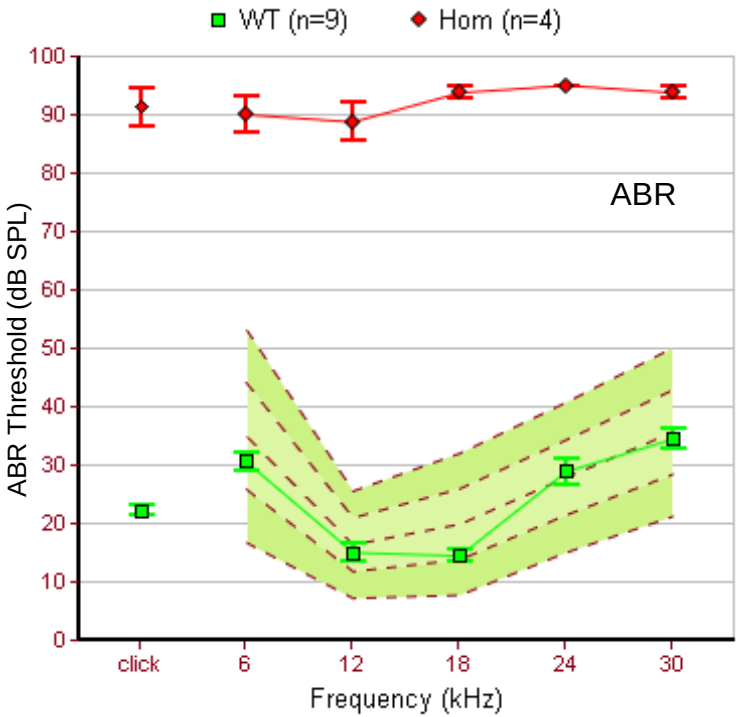
Spns2$^{tm1a(KOMP)Wtsi}$

Spinster homologue 2 (Drosophila)

- Sphingosine-1-phosphate transporter
- Pleiotropic effects including:
  - Eye Morphology
    - Eyes open at birth
    - Adult corneal opacity/vascularisation
    - Adult abnormal pupil shape/position
  - Reduced peripheral blood lymphocytes
  - Severe deafness (60-80 dB)







Maintenance

- The use of animals for the maintenance of colonies of genetically altered established lines, with a likely harmful phenotype will continue to require a project authorisation.
- The use of animals for the maintenance of colonies of genetically altered established lines without a likely harmful phenotype is not considered a procedure and thus does not require a project authorisation.

Based on Welfare Assessment

- Likely harmful phenotype
  e.g. Potential to develop Tumours

- Without a likely harmful
  e.g. Inducible GA ; Cre animals

Genotypes Compared	Colony Prefix	Allele Name	Body Weight Curve (HFD)	Dysmorphology (56)	Hair Follicle Cycling	Indirect Calorimetry (7)	Core Temperature/ Stress induced	ip-GTT (2)	DEXA (9)	X-ray Imaging (41)	Heart Weight (3)	Open Field (13)	Grip Strength (7)	Modified SHIRPA (21)	Hot Plate (2)	Eye Morphology (34)	ABR (6)	Full Clinical Chemistry (26)	Peripheral Blood Lymphocytes (9)	Haematology (10)
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EU Working Group on Severity Assessment

Directive 2010/63/EU

Article 54 on reporting:

Requires that for statistical information, the actual severity of the pain, suffering, distress or lasting harm experienced by the animal must be reported

Reasoning:

By inclusion of the actual suffering experienced by the animal, provide greater transparency and understanding of the impact of scientific procedures on animal welfare

Benefits:

Improve the quality of science and welfare.

Assessment of Severity

Examples at the EWG illustrated –

- Lack of understanding of:

Prospective assessment of what may happen to the animal

vs.

Actual severity of what really happened to the animals

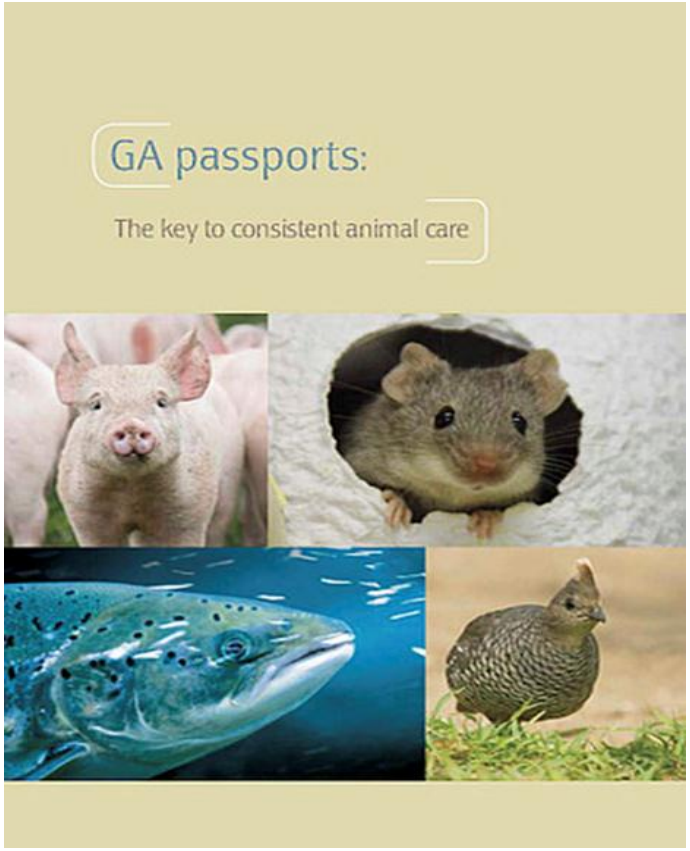
Discussions Highlighted –

- Massive diversity in interpreting mutation effect, phenotype & experimental impact, background and ameliorating factors.
- Initial interaction between many parties (NACWO, PiL, NVS etc)
- Consistent Assessment – No short term answer but should be a long term aspiration?

Promoting Consistency

- Common understanding of mutation impact
- Use of Health/welfare information – Mouse Passport
- Effective communication
- Ameliorate adverse effects
- Inform for greater refinement where possible

Transgenic Technologies Working Group



wellcome trust sanger institute

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Ninj2 (MEQF EPD0524_3_D04)

Allele: *Ninj2*$^{tm1a(KOMP)Wtsi}$

Embryonic stem cell targeted: JM8A3.N1
Embryonic stem cell origin: C57BL/6N-A^{tm1Brd}/a
Background used for Germ Line Transmission: C57BL/6N Taconic USA
Subsequent backcross background: Inter cross from within Colony
Genetic background: C57BL/6N Taconic USA; C57BL/6N-A^{tm1Brd}/a

Coat Colour Information:

Agouti and Black

Breeding Performance and Lifespan:

- Generally heterozygous mice from this colony conform to normal expectations of the background strain.
  For maintenance of our colonies we pay particular attention to the age of the mating pairs and the resulting litters. In our experience the C57BL/6N substrain used to establish and progress this colony has shown some characteristics such as poor breeding, high pre-weaning mortality rates and failure to breed beyond three litters. We believe disturbance of litters has a detrimental effect on the mating pair. For our core and mutant colonies we have actively reduced our intervention with the mice. Daily observations, health checks, cleaning and cage movement is minimised in litters under 14 days of age.
- Homozygous Viable.

Bedding:

Aspen Chip derived from a Baltic supply – Supplier B&K Universal

Diet:

Autoclavable Mouse Breeder Diet 5021 – A controlled constant-nutrient diet formulated to compensate for nutrient losses that occur during steam sterilization. Supplier Lab Diet www.labdiet.com

Husbandry:

Cleaning frequency is based against cage occupancy and technician assessed level of soiling. Base changing is performed in a HEPA filtered change station which remains positive to the room environment. Gloved hands are disinfected between each cage. Diet is fed ad-libitum.

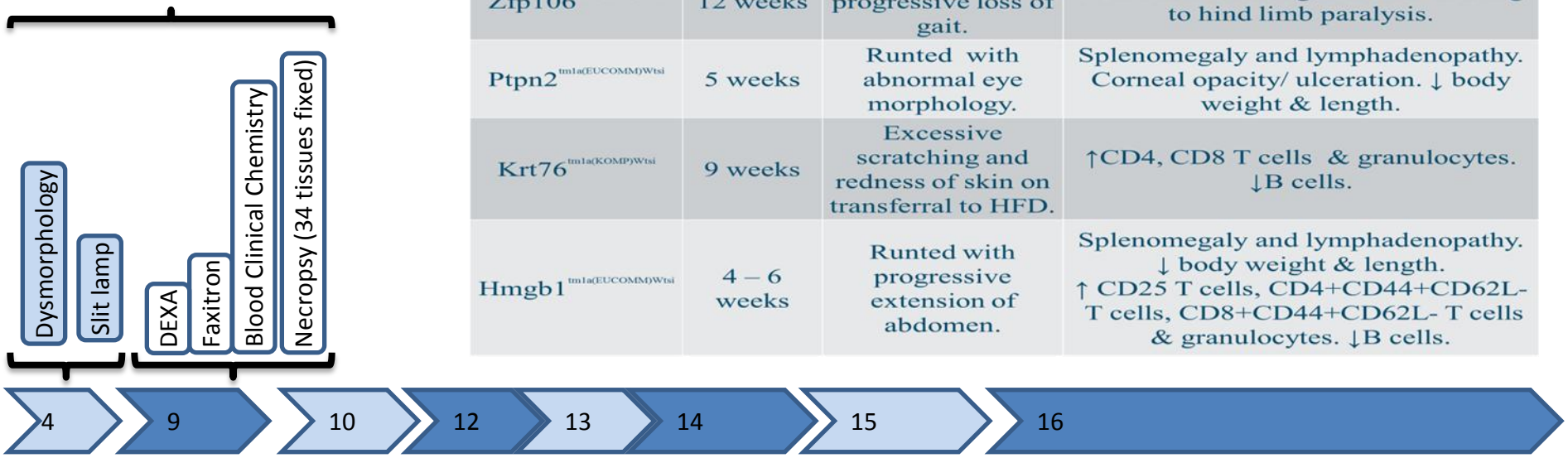
Housing System:

Individual Ventilated Cages maintained at positive pressure to the room with an average of 60 HEPA filtered air changes per hour.

Why wait till the reporting?

- Models managed to prevent moderate severity
- Technicians carryout enhanced monitoring
- Stressful or non-essential procedures removed where possible
- Experimental procedures terminated prior to onset of phenotype

Modified phenotyping pipeline



Allele	Homs terminated	Welfare observation	Phenotypes reported
Zfp106$^{tm1a(KOMP)Wtsi}$	12 weeks	Hunched, with progressive loss of gait.	Neuromuscular degeneration leading to hind limb paralysis.
Ptpn2$^{tm1a(EUCOMM)Wtsi}$	5 weeks	Runted with abnormal eye morphology.	Splenomegaly and lymphadenopathy. Corneal opacity/ ulceration. ↓ body weight & length.
Krt76$^{tm1a(KOMP)Wtsi}$	9 weeks	Excessive scratching and redness of skin on transferral to HFD.	↑CD4, CD8 T cells & granulocytes. ↓B cells.
Hmgb1$^{tm1a(EUCOMM)Wtsi}$	4 – 6 weeks	Runted with progressive extension of abdomen.	Splenomegaly and lymphadenopathy. ↓ body weight & length. ↑ CD25 T cells, CD4+CD44+CD62L- T cells, CD8+CD44+CD62L- T cells & granulocytes. ↓B cells.

Application of observations within the life of the colony or experiment should allow for real time adjustment

Welfare and severity assessment conclusion:

- Assessment should drive refinement – aligned to the 3R's
- Breeding strategies adjusted according to the severity of the phenotype
- Procedures, Husbandry and Environment are adjusted accordingly
- Strategies adjusted to maximise the use of mice that can bear the procedural/genetic burden
- Human end points are redefined in light of any noted effect

Aligning this to the directive and future reporting:

- System/consistency yet to be fully applied in context to the severity assessment
- Informatics approach means this is attainable in a reasonable time frame.
- Adaptability of data capture forms aligned to the actual procedures supports easy gathering and consistent review of severity levels.

Useful links

European Commission website on EWG's

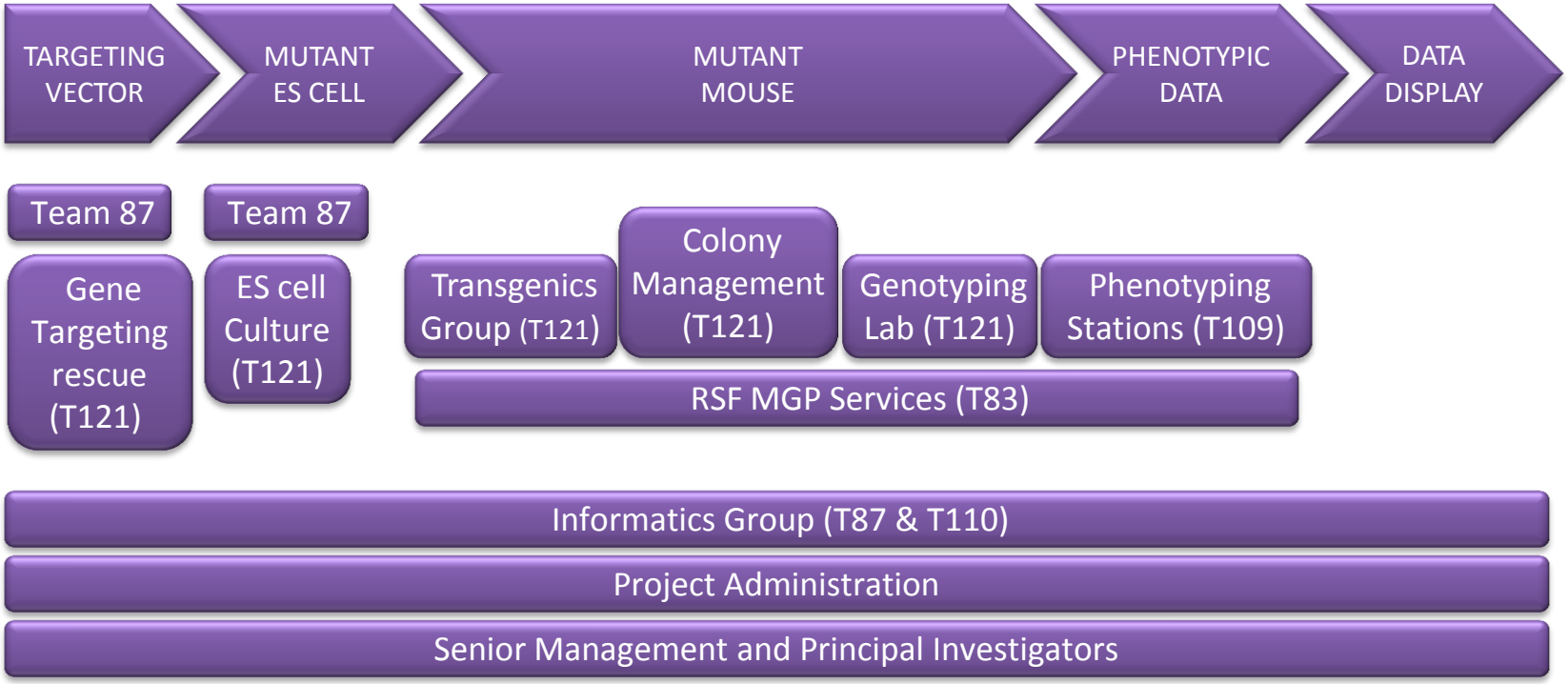
http://ec.europa.eu/environment/chemicals/lab_animals/home_en.htm

RSPCA GA Survey for TTWG

http://www.surveymonkey.com/s/GASurvey071212

Acknowledgements:

All the Sanger Mouse & Zebra Fish folk



Special thanks – David Anderson