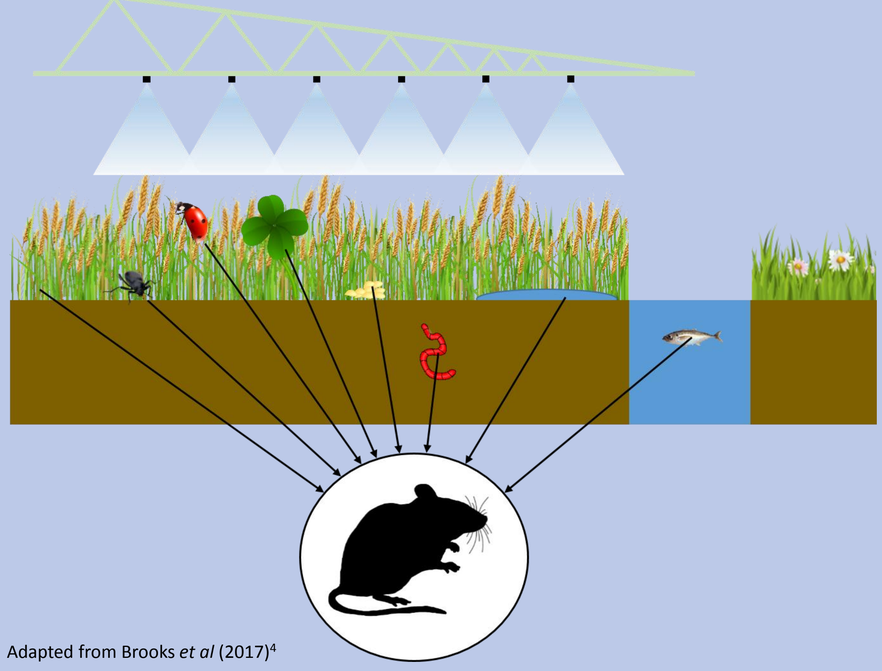
Deriving ecologically relevant endpoints for wild mammal risk assessment

Amy Brooks[1#], Steve Ruckman[1], Katharina Ott[2] & Dennis Sprenger[2]
[1]Cambridge Environmental Assessments, Boxworth, UK; [2]BASF, Limburgerhof, Germany [#]Corresponding author: amy.brooks@cea-res.co.uk

Conclusions
The lowest available no observed adverse effect level (NOAEL) in a reproductive or developmental toxicity study is not always the most appropriate endpoint to use in a reproductive risk assessment for wild mammals. Effects deemed relevant for human risk assessment may not be relevant for wild mammal populations. All relevant mammalian data should be used to derive an ecologically relevant endpoint, taking into account the type, magnitude and consistency of effects observed and contextualising them in terms of natural variability. The exposure profile used in toxicity studies should also be considered, as this may lead to an overestimation of effects. Benchmark dose modelling may also be helpful, but realistic agreed benchmark response levels are still lacking.



Adapted from Brooks *et al* (2017)[4]

Why do we need a chronic mammalian endpoint?
Wild mammals living and feeding in agricultural habitats are potentially exposed to plant protection products (PPPs), used to protect crops from various pests and diseases.

The protection goals of the risk assessment guidance[1] are to clearly establish no visible mortality and no long term repercussions for abundance and diversity of wild mammal populations, making effects upon mortality or reproduction unlikely.

Tier I risk assessments use the lowest available NOAEL derived for human health risk assessment taken from the developmental study or 2-generation rat study, regardless of ecological relevance for wild mammal populations.

Some guidance exists[1,2] regarding which *types* of effects are considered relevant for wild mammal populations, but little on how to determine their ecological relevance; instead, expert judgement is required.

Member State experts[3] recently recommended that ecologically relevant reproductive endpoints should be assessed and agreed during active substance evaluations at EU level rather than during national registrations. To aid this process, the main factors that should be considered are outlined below, based upon our experiences to date:

Type of effects observed
- Often no effects on reproductive endpoints (e.g. litter size, pup survival) but slight effects on pup body weight.
- Reduced pup weight may not be linked to reduced reproductive success in adulthood, so relevance to reproductive risk assessment is questionable, e.g. F1 pups with reduced body weight observed to have normal litter size (F2)
- Adverse vs adaptive changes, e.g. reduced body weight may be due not directly to toxicity but to unpalatability of treated diet or to metabolic and/or hormonal stresses induced by exposure to the test material

Benchmark dose (BMD) modelling
- Approach strongly recommended by EFSA working group
- Less subjective than using NOAELs
- New BMD programmes available to standardise running, reporting and interpretation of models
- BMD approach useful where wide dosing interval and therefore the NOAEL is artificially low due to dose spacing
- Currently a lack of agreed and realistic benchmark response (BMR) levels in terms of at what magnitude an effect is considered to be population relevant

Consistency of dose-response patterns
- The same effects may not be seen throughout the study and therefore may be transient – e.g. a slight body weight effect observed in F0 females and F1 pups but not in F1 adults or F2 pups
- The same effect may not be seen in other available toxicology studies using similar doses, such as the developmental studies in rats and rabbits or in the general toxicity studies with similar treatment periods (e.g. 28- or 90-day studies in rats, mice or dogs)
- Studies undertaken using similar doses but different routes of exposure (dietary vs gavage) can be useful to examine whether reduced body weight is an adverse effect or may be related at least in part to lower food intake resulting from unpalatability of the test item, which is less relevant for wild mammals with alternative food sources
- All relevant studies should be considered and an overview of observed effects taken, such that a NOAEL is not derived due to the dose spacing used in a single study e.g. a NOAEL of 50 mg/kg bw/d instead of 25 mg/kg bw/d in the illustration below:

Study / dose	25 mg/kg bw/d	50 mg/kg bw/d	100 mg/kg bw/d	200 mg/kg bw/d
2-generation rat study	No effect	*(Not tested)*	**Effect**	**Effect**
Rat development study	*(Not tested)*	No effect	**Effect**	**Effect**
Rabbit development study	*(Not tested)*	No effect	No effect	**Effect**

What factors should we consider when deriving an ecologically relevant endpoint?

Magnitude of effects observed
- Statistical significance doesn't necessarily equate to toxicological or ecological relevance
- Contextualise results using natural variability in wild populations e.g. body weight in wild rodents can vary by as much as 42%; therefore small fluctuations in body weight are unlikely to have significant impacts on breeding behaviours
- Historical control data can be used to contextualise effects seen in laboratory animals – e.g. if body weights statistically significantly different from study controls are within historical control ranges, it is questionable whether such effects would be of biological significance especially given the range of normal variation in bodyweight in the wild
- Effects can be exaggerated by natural variability e.g. variation in growth rate from one generation to the next and variation in litter size between mothers, which in turn may inversely impact upon individual pup weights

Relevance of exposure profiles
- Exposure across multiple generations experienced in laboratory study may be unrealistically prolonged compared to likely exposure of wild mammals e.g. seed treatments occur once at start of growing season, followed by residue degradation/dissipation and also germination within 1-2 weeks; active substance may also be rapidly metabolised/excreted by mammals → exposure of multiple generations may not occur
- Delayed onset of effects within a generation may not be relevant if required exposure duration unlikely from proposed uses e.g. reduction in body weight not observed until week 5 in F0 females and so unlikely to occur from single application on crop (as growth effects usually attributable to long term exposure)
- A bodyweight effect upon suckling offspring in a laboratory multi-generation study may be associated with an impaired nutritional quality of the mother's milk, resulting from a prior prolonged exposure of the mother, rather than to a direct effect upon the neonate
- Dose experienced by offspring when they start eating the treated diet is higher than for adults e.g. a nominal 85 mg/kg bw/d for adults vs >300 mg/kg bw/d for pups and young premating adults → higher effects may be due to dose not being adjusted for body weight of the pup, rather than the adult

References: [1]EFSA (2009) Bird and mammal guidance. EFSA Journal, 7, 1438 [139 pp.];[2]PPR opinion (2006). EFSA Journal, 344, 1-22; [3]EFSA supporting publication 2015: EN-924. 62 pp.; [4]Brooks *et al* (2017) ET&C,36(3), 565-575