Agrochemical exploratory toxicology
Integrating mechanistic toxicology into candidate selection
CXR Biosciences®: exploratory toxicology

Exploratory toxicology studies are those carried out prior to selection of a development candidate. It follows that the primary aim of exploratory toxicology is to identify development candidates with the optimal toxicological properties. This includes ensuring that acute toxicity thresholds suggested by regulatory guidance are likely to be met. Two additional aims are (i) to predict the toxicities likely to be seen in regulatory toxicity studies, in order to ensure that suitable mechanistic endpoints can be included in those studies, and (ii) to use toxicokinetic (TK) data early in a testing program to understand the relationship between dose and systemic exposure.

CXR Biosciences® has an extensive track record in exploratory toxicology, having supported the programs of leading agrochemical companies for many years. We understand the scientific and industry context in which the studies are performed, and our flexible scheduling means we can deliver quality data in the short timeframes demanded by an agrochemical research organisation.
Aims of exploratory toxicology

Aim 1: use short-term exploratory toxicity studies, in vitro mechanistic screens and in vitro comparative ADME to choose the optimal development candidate.

- Studies are designed to maximise data generated with limited compound availability, e.g. CXR’s microsampling TK approach means no satellite TK groups are required. Specific class-related mechanistic endpoints can be tested in parallel to aid selection.
- CXR’s flexible schedules allows rapid identification of those candidates that meet suggested toxicity thresholds.
- Cross-species differences in rates of metabolism and metabolites formed can be defined early in the testing program using in vitro studies, in order to assist in the interpretation of toxicity data and inform design of subsequent studies in rodents and other species.

Aim 2: predict toxicities likely to be seen in sub-chronic and chronic regulatory studies, in order to be able to ensure that suitable mechanistic endpoints are included in these regulatory studies.

- For example, if the primary target organs of toxicity can be identified as early as possible in the testing program (e.g. in the first repeat-dose exploratory studies), samples of those organs can be taken and retained for potential mechanistic investigation from subsequent regulatory studies.
- The target organs can be used for explorations of potential mechanisms of toxicity using appropriate biochemical or genomic assays.
- This can obviate the need for later standalone mechanistic studies after the regulatory toxicity studies have been completed, leading potentially to more rapid product launch, as well as being desirable from an animal usage (3Rs) perspective.

Aim 3: use toxicokinetic data early-on in a testing program to understand the relationship between dose and systemic exposure, and to identify potential inflection point doses for onset of non-linear toxicokinetic behaviour.

- Early TK data can be very useful in selecting doses for later toxicity studies that avoid the potential for unnecessary generation of high-dose specific (Maximum Tolerated Dose, MTD) toxicity that occurs only at dose(s) in the non-linear TK dose range.
- If doses defining the inflection point for onset of TK saturation are well separated from human exposures, OECD guidelines¹ allow for top dose selection that is at or only slightly above the inflection point for onset of non-linearity (Kinetically-Derived Maximum Dose, KMD²).
- Since it is not unusual in pesticide toxicity testing to see toxicity which is limited to only the highest dose tested (MTD), any reasonable option to reduce the top dose level below a conventional MTD reduces the potential for generation of a “false positive” high-dose result that has no human relevance. This is specifically true for agrochemical active substances, as expected human exposure through environment/residue in food is usually in ng/kg/day range and does not usually necessitate testing such high doses.
- At least one major agrochemical company currently employs the KMD approach to all its development compounds in order to facilitate rational dose selection strategies for its registration toxicity studies.

¹For example, OECD/OCDE 443: OECD Guideline For The Testing Of Chemicals – Extended One-Generation Reproductive Toxicity Study.
²References include Creton et al., Regulatory Toxicology and Pharmacology, Volume 62, Issue 2, March 2012, Pages 241–247; Saghir et al., Regulatory Toxicology and Pharmacology Volume 63, Issue 2, July 2012, Pages 321–332; Terry et al., Critical Reviews in Toxicology Volume 44 S2, 2014, Pages 1–14. It may not be possible to identify a KMD until a 28-day study is performed.
Exploratory toxicology differs greatly from the regulatory toxicology studies that are conducted once a candidate development compound is selected (Figure 1).

In the regulatory studies, a single compound is empirically characterized in a series of defined studies over a number of years.

In exploratory toxicology, only the biological target\(^3\) is constant, and lead chemistry constantly evolves over a short timeframe, requiring rapid generation of data to drive further chemical design, and ranking and de-selection of compounds.

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Exploratory Toxicology, for example:
- Acutes & repeat dose
- Toxicokinetics
- Mechanistic in vitro
- In vitro metabolism

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\(^3\)In contrast to the situation in the pharmaceutical industry, the biological target is frequently not present in mammalian species, but only in the target organism. So the classic “on-target” toxicity seen in pharmaceuticals is less likely to be a concern, although mammalian toxicity may be driven by interactions with biologically-similar targets.
Exploratory toxicology – the CXR approach

CXR Biosciences® has an extensive track record in exploratory toxicology, having supported the programs of leading agrochemical companies for many years. We understand the scientific and industry context in which the studies are performed, and our flexible scheduling means we can deliver quality data in the short timeframes demanded by an agrochemical research organisation.

CXR can support entire programs by:

- Reviewing potential (e.g. on-target) or observed toxicity issues
- Developing an hypothesis and consequent experimental programme
- Performing the required experiments
- Understanding translational risk assessment, and driving chemical design and / or candidate selection

A typical exploratory toxicology program will have two to four key components (Figure 2):

1. First-in-animal acute toxicity studies
2. Repeat dose 7, 14 or 28 day studies
3. If the biological target warrants it, mechanistic in vitro screens for potential mechanisms of concern
4. Cross-species in vitro metabolism

*Many of our larger customers use CXR on a study-by-study basis when internal exploratory resources are stretched.

Fig 2. A Typical Exploratory Toxicology Program

1. Acute, escalating dose rat study
   **Aim:** Identify acute toxicities, select doses for repeat dose study
   - Oral admin
   - 24 hour to 7 day obs
   - TK measurements using CXR microsampling approach
   - Optional metabolite identification and mass balance
   - Optional mechanistic endpoints (e.g. gene expression)

2. 7–28 day rat exploratory tox study
   **Aim:** to compare candidates, select doses for longer-term regulatory studies.
   - If possible estimate KMD, identify dose at which nonlinear TK begins
   - Oral and/or dietary admin (based on hypothesised MOA, and therefore if AUC or Cmax the key parameter)
   - 1–3 dose levels (limited by compound availability)
   - TK day 1 and day last using CXR microsampling approach
   - Optional metabolite identification and mass balance
   - Optional mechanistic endpoints e.g. tyrosine, GADD45
   - Slides for H&E
   - Key tissues stored for possible later mechanistic investigation (e.g. toxicogenomics, liver enzyme levels)

3. Relevant target-dependent mechanistic screens, e.g.
   - Endocrine disruption (e.g. CYP 19/51)
   - Mitochondrial dysfunction
   - Oxidative stress
   - Tubulin (de) polymerisation
   - Binding to cerebral membranes
   - Rat hepatocyte induction / proliferation screening

4. Cross species metabolism in hepatocytes
   - Rates, metabolite identification
   - Drives study design in rodents, dog, rabbit
1 Acute Studies Aims:

- Identify acutely toxic compounds; meet dose criteria for toxicity.
- Understand relationship between single doses and systemic exposure.
- Select doses for repeat dose studies, such that compounds can be compared on the basis of systemic exposure rather than dose.

Typical designs:

- 1–6 single dose levels, typically by gavage.
- 3–5 rats per group.
- Clinical observations and body weights, 48 hours to 7 days.
- TK samples taken using CXRs microsampling technique (no satellite animals required) – for example 14 timepoints over 7 days, analysed for active ingredient and potentially for metabolites.
- Optional mechanistic markers, for example Taqman for gene expression, levels of key markers (e.g. tyrosine).

2 Repeat Dose Studies Aims:

- Compare toxicities of potential candidates.
- Understand relationship between repeat doses and systemic exposure.
- If possible identify dose at which non-linear kinetics appears (i.e. Kinetically-derived Maximum Dose, or KMD).

Typical designs:

- 1–3 repeat dose levels, typically by gavage.
- 5 rats per group, one or both sexes.
- Clinical observations and body weights.
- Histopathology on key target organs.
- TK samples taken using CXRs microsampling technique (no satellite animals required) – for example TK profile taken on day 1, and day last.
- Optional mechanistic markers, for example Taqman for gene expression, levels of key markers (e.g. tyrosine).
- Key tissues stored for potential mechanistic investigations (e.g. microarray analysis, microsome preparation for analysis of CYP levels).

3 Mechanistic in vitro screens Aims:

- Compare compounds for their ability to modulate toxic mechanisms of potential concern.

Example screens:

- Autoinduction and non-genotoxic carcinogenicity risk. Screen multiple concentrations of test items for their ability to cause CYP induction in rat hepatocytes. 96 well format, Taqman endpoint. Identifies potential autoinduction and non-genotoxic carcinogenicity issues.
Summary

- Exploratory toxicology studies are those carried out prior to selection of a development candidate.
- The primary aims of exploratory toxicology are:
  - to drive the selection of the development candidate with the optimal toxicological properties, and to meet acute toxicity criteria.
  - to predict the toxicities likely to be seen in later regulatory studies.
  - use toxicokinetic data to understand the relationship between dose and systemic exposure, and if possible to identify potential inflection point doses for onset of non-linear toxicokinetic behaviour.
- CXR Biosciences has an extensive track record in exploratory toxicology, having supported the programs of leading agrochemical companies for many years.
- We understand the scientific and industry context in which the studies are performed.
- Our flexible scheduling means we can deliver quality data in the short timeframes demanded by an agrochemical research organisation.

- Endocrine disruption.
  Antifungal CYP51 (lanosterol 14-demethylase) inhibitors can also inhibit human CYP51 and CYP19 (aromatase), an unwanted side effect that may cause estrogen biosynthesis reduction and endocrine disruption. CXR offers in vitro assays to assess whether your compound inhibits human or rat CYP19 or CYP51.

- Endocrine disruption potential as measured by androgen and/or estrogen receptor transactivation.

- Mitochondrial dysfunction.
  Some biological targets (e.g. chloroplast related) may carry an increased risk of causing mitochondrial dysfunction. CXR can screen for mitochondrial dysfunction (via e.g. ATP concentration, respiration and respiratory control, oxygen consumption, mitochondrial membrane potential, mitochondrial swelling, reactive oxygen species and GSH concentrations, fatty acid beta-oxidation, apoptosis).

- Effects of tubulin binders on mammalian tubulin (tubulin polymerisation and depolymerisation). Fresh porcine tubulin is treated with test items and positive control compounds taxol and colchicine.

- Effects on mammalian cholinesterase activity. Assessed by testing for affinity to the rat vesicular acetylcholine transporter, by ex vivo competition radioligand binding on rat cerebral membranes, vesamicol as control.

- Oxidative stress. Oxidative stress is linked to a number of toxicities, and the activation of the Antioxidant Response Element in the AREc32 cell line provides a screen to identify potentially toxic compounds that induce oxidative stress.

- CXR will establish other mechanistic in vitro assays on request.

4 In vitro metabolism

A full understanding of the metabolism of compounds is required in the biological systems to which the product will be exposed (for example as mandated by Regulation EC 1107/2009 for agrochemicals). Prior to this, in vitro metabolism studies can also be used to select the most appropriate preclinical species for regulatory studies, and to aid in the design and interpretation of those studies, by comparing rates of metabolism and metabolites formed in different species. We routinely run comparative metabolism studies using hepatocytes or microsomes from a range of species (for example mouse, rat, dog, human, rabbit, others as required) to demonstrate comparability of metabolism kinetics and to screen for species-specific major metabolites.
Founded in 2001, CXR Biosciences® uses its collaborative approach and toxicological expertise to help customers of all sizes solve issues relating to the safety of compounds or selection of chemical or drug candidates. CXR Biosciences® offers tailored preclinical services in the areas of investigative and mechanistic toxicology, exploratory and discovery toxicology and PK & metabolism. Our customers include leading agrochemical, chemical, pharmaceutical, biotechnology and consumer product companies. CXR Biosciences® is located in Dundee, Scotland, United Kingdom.

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