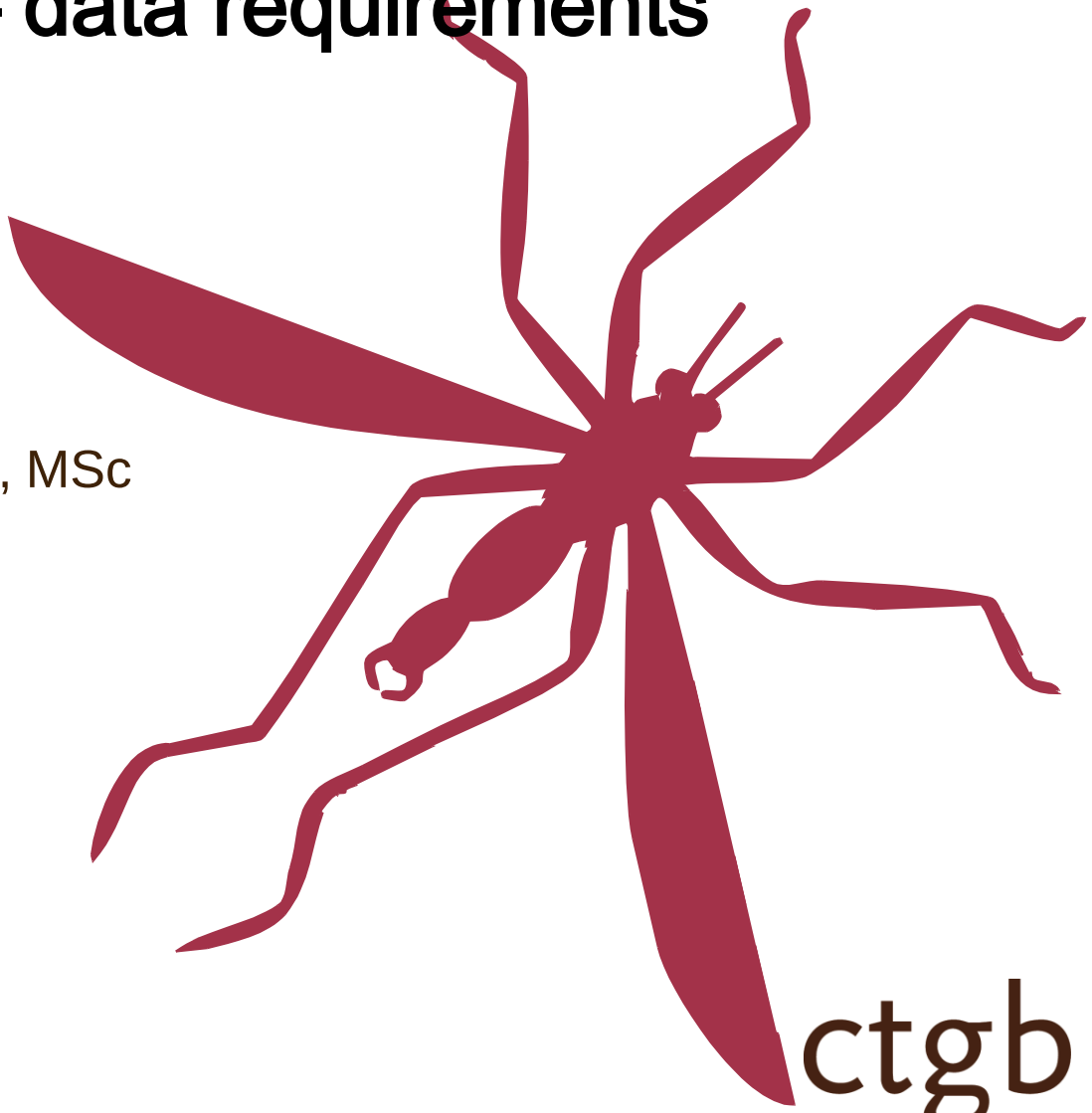
General framework of toxicology evaluation – data requirements



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EU framework (Regulation (EC) 1107/2009)

Data requirements

- Active substance: Commission Regulation (EU) No 544/2011
- Product: Commission Regulation (EU) No 545/2011

- “The information provided must be sufficient to permit an evaluation to be made as to the risk for man, associated with the handling and use of plant protection products containing the active substance, and the risk for man arising from residual traces remaining in food and water.”

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EU framework (Regulation (EC) 1107/2009)

- In addition, the information provided must be sufficient to:
  - establish a relevant acceptable daily intake (ADI) level for man,
  - establish acceptable operator exposure level(s) (AOEL),
  - specify the pictograms, signal words, and relevant hazard and precautionary statements for the protection of man, animals and the environment to be included in packaging (containers),
  - identify relevant first aid measures as well as appropriate diagnostic and therapeutic measures to be followed in the event of poisoning in man.

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EU: Data requirements active substance

- Toxicokinetics
- Acute toxicity
- Short-term toxicity
- Sub-chronic toxicity
- Genotoxicity testing
- Long-term toxicity and carcinogenicity
- Reproductive toxicity
- Delayed neurotoxicity studies
- Other toxicological studies
- Medical data

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Toxicokinetics

- Limited data in one species (rat)
  - Rate and extent of absorption
  - Tissue distribution and excretion
  - Identification of metabolites and metabolic pathway
- Always required

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Acute toxicity (oral, dermal, inhalation)

- Basis for classification and labelling, and estimating toxicity ranges.
- Animals:
  - oral/inhalation; rat
  - Dermal; rat, rabbit or guinea pig
  - ≥ 5/sex/dose level
- Dose: ≥ 3 levels or limit dose (2000 mg/kg bw or 5 mg/l for 4 h)
- Dermal and oral always required, inhalation required if use will result in respirable material

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Acute toxicity (oral, dermal, inhalation)

- Observations (≥14 days)
  - Mortality, clinical signs daily
  - Body weight day 0, 3, 7, 14
  - Necropsy (macroscopy)

- Results: LD50 values → classification
  - E.g if LD50 oral rat (mg/kg bw)

Below 50	Fatal
Between 50 and 300	Toxic
Between 300 and 2000	Harmful

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Acute toxicity – skin irritation

- Always required except if:
  - Active substance has predictable corrosive potential (e.g. pH)
- Animals: rabbit, n=3
- Application: 6 cm^2, 0.5 ml/0.5 g, with semi-occlusive dressing, 4 hours
- Observations:
  - 1, 24, 48, 72 hrs after patch removal
  - Grade erythema + oedema

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Acute toxicity – eye irritation

- Always required, except if :
  - severe reaction is expected based on physico-chemical properties (e.g. pH)
- Animals: rabbit, n = 3
- Application: 0.1 ml/100 mg in conjunctival sac
- Observations: grade changes cornea, iris, conjunctivae redness and chemosis at 1, 24, 48 and 72 hrs after application

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Acute toxicity – Skin sensitisation

- Always required, except if substance is a known sensitiser.
- LLNA or Guinea Pig Maximisation Test (preferred over Buehler)
- Animal (Maximisation): guinea pig, n=10 treatment, n=5 control
- Treatment: induction on day 0 (intradermal) and 7 (topical), challenge on day 21 (topical)

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Sub-acute (28-day) oral toxicity

- Not mandatory: useful as range finding tests.
- Animals: rat, ≥ 5/sex/dose
- Observations: mortality, clinical observations, bw, food consumption, haematology, clinical chemistry, urinalysis, organ weight, necropsy, histopathology
- Endpoint: evaluation of all results → NOAEL

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Sub-chronic (90-day) oral toxicity

- Always required
- Animals: rat and dog
  - If the dog is significantly more sensitive than a 12-month study in dogs may be required
- Observations: similar to sub-acute study + ophthalmoscopy and more extensive histopathology

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Genotoxic toxicity testing

- Required: *In vitro* studies (bacterial assay for gene mutation, mammalian cells assay for clastogenicity and gene mutation)
- *In vivo* studies in somatic cells, required if:
  - the *in vitro* tests are negative.
  - the *in vitro* cytogenetic test is positive (metaphase analysis in rodent bone or micronucleus in rodents).
  - either of the *in vitro* gene mutation test are positive (unscheduled DNA synthesis or a mouse spot test).
- *In vivo* studies in germ cells, required if:
  - any of the *in vivo* study in somatic cells is positive.

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Chronic (2-years) toxicity testing

- Always required
  - Can be combined with carcinogenicity study
- Animals: rat
- Observation: same as sub-chronic + haematology and urinalysis at 6 months.

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Carcinogenicity

- Always required
- Purpose: to identify carcinogenic effects resulting from exposure
- Animals: rat and mouse,
- ≥ 50/sex/dose
- Observation: mortality, clinical signs, tumors, bw, food consumption, necropsy, histopathology

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Reproductive toxicity

- Always required
- Multi-generation study: over ≥ 2 generations
- Animals: rat, 20 litters/dose
- Observations: clinical signs, bw, food consumption, necropsy, fertility, litter data (e.g. pup size, still-born/dead pups)
- Endpoint:
  - NOAEL for parental toxicity
  - NOAEL for reproductive performance
  - NOAEL for development of offspring

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Developmental toxicity

- Always required
- Animals: rat and rabbit, 20 (12) pregnant rats (rabbits)/dose
- Observations: clinical signs, bw, food consumption, number of corpora lutea, implantation, resorptions, live/dead fetuses, fetal weight, malformed/abnormal fetuses, skeletal and visceral anomalies.
- Endpoints:
  - NOAEL for maternal toxicity
  - NOAEL for development of offspring
  - Teratogenicity

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Other toxicological studies

- Delayed neurotoxicity studies
  - Required for substance similar or related to those capable of inducing neurotoxicity, such as organophosphates
- Supplementary studies
  - E.g. immunotoxicological
- Medical data
  - E.g. medical surveillance on manufacturing plant personal

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EU: Data requirements product

- Acute toxicity studies
  - Oral, dermal, inhalation
  - Skin and eye irritation
  - Skin sensitisation
- Supplementary studies for combination of plant protection products (not required, case by case assessment)
- Data on operator, bystander and worker exposure (estimation or measurement)

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US framework

- Data requirements described in **Federal Register** / Vol. 72, No. 207 / Friday, October 26, 2007 / Rules and Regulations. Pesticides; Data Requirements for conventional chemicals.

- Data requirements mainly the same as those required by the EU.
  - Distinction made between food patterns and non-food use patterns (e.g. forestry use, aquatic nonfood crop use, indoor residential use).

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TABLE—TOXICOLOGY DATA REQUIREMENTS

Guideline Number	Data Requirements	Use Pattern		Test substance to support		Test Note No.
		Food	Nonfood	MP	EP	
Acute Testing						
870.1100	Acute oral toxicity - rat	R	R	TGAI and MP	TGAI, EP, and possibly diluted EP	1, 2
870.1200	Acute dermal toxicity	R	R	TGAI and MP	TGAI, EP	1, 2, 3
870.1300	Acute inhalation toxicity - rat	R	R	TGAI and MP	TGAI and EP	4
870.2400	Primary eye irritation - rabbit	R	R	TGAI and MP	TGAI and EP	3
870.2500	Primary dermal irritation	R	R	TGAI and MP	TGAI and EP	1, 3
870.2600	Dermal sensitization	R	R	TGAI and MP	TGAI and EP	3, 5
870.6100	Delayed neurotoxicity (acute) - hen	CR	CR	TGAI	TGAI	6
870.6200	Acute neurotoxicity - rat	R	R	TGAI	TGAI	7
Subchronic Testing						
870.3100	90–day Oral - rodent	R	CR	TGAI	TGAI	8, 9
870.3150	90–day Oral - non-rodent	R	CR	TGAI	TGAI	36
870.3200	21/28–day Dermal	R	NR	TGAI	TGAI and EP	10, 11
870.3250	90–day Dermal	CR	R	TGAI	TGAI and EP	11, 12
870.3465	90–day Inhalation - rat	CR	CR	TGAI	TGAI	13, 14
870.6100	28–day Delayed neurotoxicity-hen	CR	CR	TGAI	TGAI	6, 15
870.6200	90–day Neurotoxicity - rat	R	R	TGAI	TGAI	7, 16

CR = conditionally required

NR = not required

R = required

EP = endpoint product

MP = manufacturing-use product

TGAI = technical grade of the active ingredient

PAIRA = pure active ingredient radio-labeled

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TABLE—TOXICOLOGY DATA REQUIREMENTS—Continued

Guideline Number	Data Requirements	Use Pattern		Test substance to support		Test Note No.
		Food	Nonfood	MP	EP	
Chronic Testing						
870.4100	Chronic oral - rodent	R	CR	TGAI	TGAI	17, 18, 19
870.4200	Carcinogenicity - two rodent species - rat and mouse preferred	R	CR	TGAI	TGAI	9, 17, 18, 19, 20, 21
Developmental Toxicity and Reproduction						
870.3700	Prenatal Developmental toxicity - rat and rabbit, preferred	R	R	TGAI	TGAI	22, 23, 24, 25, 26
870.3800	Reproduction and fertility effects	R	R	TGAI	TGAI	26, 27, 29
870.6300	Developmental neurotoxicity	CR	CR	TGAI	TGAI	27, 28, 29
Mutagenicity Testing						
870.5100	Bacterial reverse mutation assay	R	R	TGAI	TGAI	30
870.5300 870.5375	*In vitro* mammalian cell assay	R	R	TGAI	TGAI	30, 31
870.5385 870.5395	*In vivo* cytogenetics	R	R	TGAI	TGAI	30, 32
Special Testing						
870.7485	Metabolism and pharmacokinetics	R	CR	PAI or PAIRA	PAI or PAIRA	33
870.7200	Companion animal safety	CR	CR	NR	TGAI or EP	34
870.7600	Dermal penetration	CR	CR	Choice	Choice	35
870.7800	Immunotoxicity	R	R	TGAI	TGAI	

CR = conditionally required
NR = not required
R = required
EP = endpoint product
MP = manufacturing-use product
TGAI = technical grade of the active ingredient
PAIRA = pure active ingredient radio-labeled

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Test guidelines

- EU: Regulation (EC) No 440/2008
- US: EPA harmonized test guidelines
- Both EU and US guidelines are equivalent to the OECD guidelines for toxicity testing.

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Quality check

Studies should be performed according to:

- standard test protocol (EC/OECD=validated)
- GLP (Good Laboratory Practice

Public (peer reviewed) literature often does not fulfill standard requirements, but can give additional information

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References

- EU Regulation (EC) 1107/2009 http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2009:309:0001:0050:EN:PDF
- EU Commission Regulation (EU) 544/2011 http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2011:155:0001:0066:EN:PDF
- EU Commission Regulation (EU) No 545/2011http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2008:142:0001:0739:EN:PDF
- Regulation (EC) No 440/2008 http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2008:142:0001:0739:EN:PDF
- US framework: http://www.gpo.gov/fdsys/pkg/FR-2007-10-26/pdf/E7-20826.pdf
- EPA guidelines: http://www.epa.gov/ocspp/pubs/frs/publications/Test_Guidelines/series870.htm
- OECD guidelines: http://www.oecd-ilibrary.org/environment/oecd-guidelines-for-the-testing-of-chemicals-section-4-health-effects_20745788

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