Food additives used in foods for infants below 16 weeks of age: additional information from animal tests

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Test substance in food intended for infants below 16 weeks of age

The additional information needed for substances added to food for infants below the age of 16 weeks depends on:

- **ADME** studies
  - substance **not absorbed** in relevant amounts
  - substance or its metabolite(s) **absorbed**

Information from standard toxicity tests

No adverse effects

- 28-day and 90-day toxicity studies (OECD 407, 408)
- Carcinogenicity and chronic toxicity studies (OECD 451, 452, 453)

Reproduction toxicity studies

Studies	OECD number	Date adopted
Prenatal developmental toxicity study	414	25 June 2018
One-generation reproduction toxicity study	415	26 May 1983
Two-generation reproduction toxicity study	416	22 January 2001
Reproduction/developmental toxicity screening test	421	28 July 2015
Combined repeated dose toxicity study with the reproduction/developmental toxicity screening test	422	29 July 2016
Developmental neurotoxicity study	426	16 October 2007

Extended One Generation Reproduction Toxicity Study (EOGRTS)

Study design EOGRTS (OECD 443) adopted 25 June 2018

	Dosing				Necropsy P animals
	Pre-mating	Mating	Post-mating		
P Males	2 weeks	2 weeks	6 weeks		
P Females	2 weeks	2 weeks	Pregnancy	Lactation	
F1			In-utero development	Pre-weaning	Post-weaning

Parental generation	Cohort	Designation	Animals / cohort	Sexual maturation	Approximate age at necropsy (weeks)
	1A	Repro	20M + 20F	Yes	11-12
Target is	1B	Repro	20M + 20F	Yes	14 or 20-25 if triggered
20 litters	2A	Neuro	10M + 10F	Yes	9
per group	2B	Neuro	10M + 10F*	No	3
	3	Immuno	10M + 10F*	Yes	8
	Surplus	Spares		No	3

Extended One Generation Reproduction Toxicity Study (EOGRTS)

EOGRTS – use of ADME data

Newly adopted guideline stresses to use existing knowledge

No or poor absorption, no reproduction toxicity study needed

ADME data in pregnant animals as part of dose-range finding (eg. in OECD 421)

- in utero exposure (via placental transport)
- via milk (sufficiently high exposure?)

Use of ADME data for selection of dose levels

Extended One Generation Reproduction Toxicity Study (EOGRTS)

Additional parameters in comparison to two-generation reproductive toxicity study parameters

F0-generation parental animals

Necropsy:

- haematology, clinical chemistry, urine analysis
- T4+TSH hormone analysis
- organ weights and pathology of 20-25 tissues/organs
- extended ovary examination (counting follicles)

Additional observations in all F1-pups

- T4 and TSH hormone analysis PND 4
- anogenital distance (once between PND 0-4)
- nipple retention (male pups on PND 12 or 13)

Necropsy at PND 21 (10/sex)

- T4 and TSH hormone analysis PND 21
- organ weights (3 organs) and pathology (10 organs)

Extended One Generation Reproduction Toxicity Study (EOGRTS)

EOGRTS – cohort 1A (reproductive)

Primary assessment of effects upon reproductive systems and of general toxicity

- *sexual maturation*, onset first cornified oestrous smears

Necropsy:

- haematology and clinical chemistry, T4+TSH hormone analysis,
- organ weights and pathology of 20-25 tissues,
- extended ovary examination (counting follicles)

EOGRTS – cohort 1B (reproductive)

Effects upon reproductive systems and of general toxicity; used for mating and produce F2-generation if triggered or planned

- *sexual maturation*

Necropsy:

preservation of organs and pathology in case of equivocal results in cohort 1A

Extended One Generation Reproduction Toxicity Study (EOGRTS)

EOGRTS – cohort 2A (neurotoxicity)

-sexual maturation

Neurodevelopmental endpoints and neurohistopathology in young adult animals

- Auditory startle (PND24),
- Functional Observational battery (PND 63-75), Motor Activity Assay (PND 63-75)

Necropsy:

-Neuropathology and morphometry

EOGRTS – cohort 2B (neurotoxicity)

Neurodevelopmental endpoints and neurohistopathology in pups at weaning

- Microscopy of the brains

Extended One Generation Reproduction Toxicity Study (EOGRTS)

EOGRTS – cohort 3 (immune toxicity)

-sexual maturation

Immunodevelopmental endpoints in young adult animals

On PND 56:

Animals used in a T-cell dependent antibody response assays; primary response to a T-cell dependent antigen, such as Sheep Red Blood Cells (SRBC) or Keyhole Limpet Hemocyanin (KLH)

Special considerations I

Test substance is **absorbed**:

- Presence (quantitative and qualitative) of substance or metabolites in milk should be analysed;
- When exposure via the milk is not sufficient then direct exposure of very young animals should be performed; postnatal study preferably in piglets.

Special considerations II

Test substance is **not absorbed**:

- Postnatal study preferably in piglets
  - This animal model closely resembles human anatomy, physiology and biochemistry; more than other non-rodent species
  - Gastrointestinal tract
  - Immune system

Thank you