

3.1 Reproductive toxicology: protecting the next generation

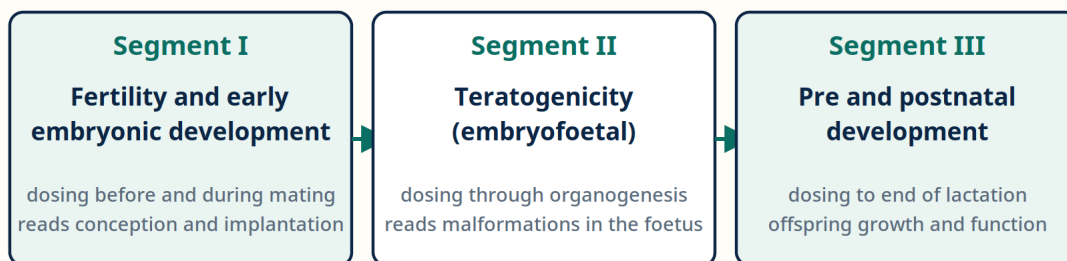
Most toxicity studies dose adult animals and watch adult organs. **Reproductive toxicology** asks what a substance does to the making of the next generation: to fertility, to the embryo and foetus developing in the uterus, and to the newborn growing to maturity. History justifies the separate discipline: thalidomide passed the general toxicity testing of its day and still produced catastrophic malformations in children, because the developing organism is not a small adult but a system under construction, in which a brief exposure on the wrong day can redirect development permanently. The PCI syllabus follows the standard international design: a three segment scheme, with male and female studies, teratogenicity at its centre.

3.2 The three segment scheme

- **Segment I** covers fertility and early embryonic development: dosing of males and females before mating and through mating (and of females into early pregnancy), reading fertility, conception, implantation and early embryo survival.
- **Segment II** covers embryofoetal development, the classical **teratogenicity study**: dosing of pregnant animals through organogenesis, the period when organs are laid down, with examination of the foetuses near term for malformations.
- **Segment III** covers pre and postnatal development: dosing of the mother from implantation through lactation, reading the birth, growth, development and eventual reproductive capacity of the offspring.

The scheme is codified in ICH S5; the OECD supplies parallel designs (TG 414 for prenatal developmental toxicity, TG 415 and 416 for one and two generation reproduction studies, TG 421 and 422 screening designs, and the extended one generation study TG 443). Examination papers use the segment language, so it is learned as printed in the syllabus.

Reproductive toxicology: the three segments



One reproductive cycle, three windows of exposure and observation (ICH S5)

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Figure: One reproductive cycle read through three windows: fertility and implantation, organogenesis, and birth to maturity.

3.3 Male reproductive toxicity studies

Male studies assess the organs and products of male reproduction under dosing: testis and epididymis weights and histopathology, sperm count, motility and morphology, and mating performance and fertility when males are paired with untreated females. Within a segment I design, males are dosed for a period covering a full spermatogenic cycle before mating (commonly at least 4 weeks, and longer in classical fertility designs), because sperm exposed as mature cells and sperm exposed as developing cells are different experiments. A substance that damages spermatogenesis may show normal mating behaviour with silently abnormal sperm, which is why the study reads both.

3.4 Segment I: fertility and early embryonic development

The segment I study doses both sexes: females from before mating through implantation, males as described above. Its endpoints trace the first links of the reproductive chain: oestrous cycling, mating and conception indices, the number of corpora lutea, implantations, resorptions and live embryos, and pre and post implantation loss. It detects effects on gametes, fertilisation and the earliest embryo that a teratogenicity study, which starts dosing after implantation, would miss entirely.

3.5 Segment II: teratogenicity and embryofoetal development

The teratogenicity study is the emotional centre of the subject. Pregnant animals, classically the rat and the rabbit as the standard second species, are dosed daily through organogenesis and the foetuses are delivered by caesarean section shortly before term, so that every foetus can be examined fresh. Findings are classified with care:

- **Malformations:** permanent structural changes harmful to the animal (the true teratogenic signal).
- **Variations:** common structural differences that are not harmful and occur in controls.
- **Growth retardation and embryofoetal death** (resorptions): developmental toxicity that is not structural.

The endpoints also separate maternal toxicity from direct developmental toxicity: a foetal effect seen only at doses that poison the mother is interpreted differently from one that appears in healthy mothers, and the developmental NOAEL is set accordingly. The rat reads the skeleton well after staining; the rabbit, being non-rodent and historically sensitive to thalidomide, is the confirmatory species.

3.6 Segment III: pre and postnatal development

Segment III doses the mother from implantation to the end of lactation and then follows the offspring, usually untreated, through weaning to maturity. It reads parturition and littering, pup survival and growth, physical and reflex development landmarks, behaviour, and in due course the reproductive performance of the F1 generation itself. It is the only segment that can detect effects expressed after birth: a substance may leave the newborn structurally normal yet alter its development or its fertility as an adult.

3.7 Endpoints across the reproductive studies

Study	Core question	Signature endpoints
Male fertility	Can treated males sire normal litters	Sperm count, motility, morphology; testis histopathology; fertility index
Segment I	Do gametes, mating and implantation work	Conception rate, implantations, pre implantation loss
Segment II	Is the embryo built correctly	Malformations, variations, resorptions, foetal weight
Segment III	Does the offspring develop and function	Pup survival, growth, landmarks, F1 reproductive performance

3.8 Genotoxicity: damage to the genetic material

Genotoxicity is damage to DNA and chromosomes: gene mutations, chromosome breaks and rearrangements, and gains or losses of whole chromosomes. It is tested separately from general toxicity because of one brutal property: a single unrepaired lesion in a single cell can, in principle, start a cancer or be passed to a child, so genotoxicity is treated as having no safe threshold by default. The regulatory question is therefore qualitative and strict: is the substance genotoxic, yes or no, answered by a battery of complementary tests rather than one assay.

3.9 The Ames test: the bacterial reverse mutation assay

The **Ames test (OECD TG 471)** is the foundation of the battery and the most examined single assay in this unit. It uses histidine dependent strains of the bacterium *Salmonella typhimurium* (with tryptophan dependent *Escherichia coli* strains in the standard set) that cannot grow without the amino acid because of a mutation in its synthesis gene. The bacteria are exposed to the test substance on minimal medium: only bacteria in which a **reverse mutation** restores the gene can form colonies, so colonies counted are mutations measured. The design has two features students must state:

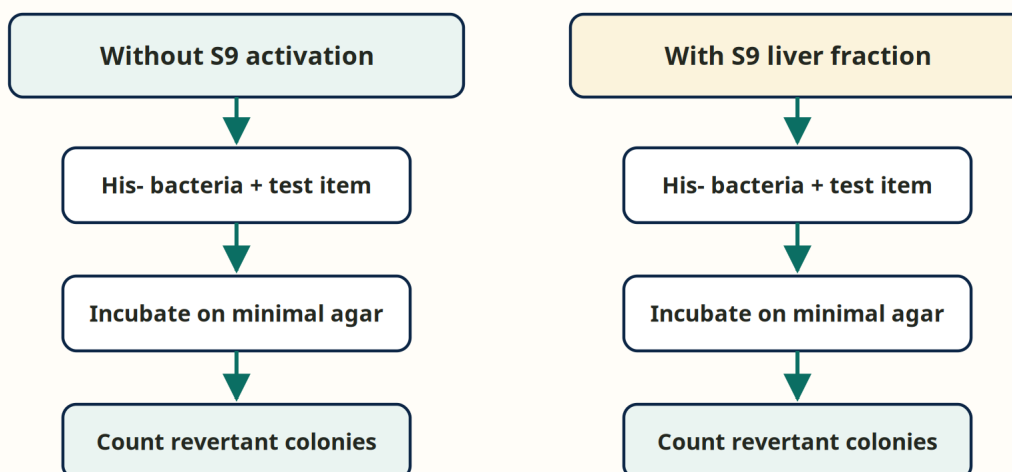
- The test is run **with and without metabolic activation**, the S9 fraction of rat liver, because many mutagens are created by metabolism rather than present in the parent substance.
- A result is positive when there is a reproducible, dose related increase in revertant colonies over the concurrent control, judged against the laboratory's historical control range, with positive controls proving the system worked.

The Ames test detects point mutations efficiently and cheaply; by design it cannot see chromosome level damage, which is why the battery continues.

The standard genotoxicity battery (ICH S2(R1), option 1)

```
Ames test (TG 471) ..... gene mutations in bacteria
+
In vitro mammalian test ..... chromosomal damage or mutation
(chromosomal aberration TG 473,      in cultured cells
or in vitro micronucleus TG 487)
+
In vivo test, normally the ..... chromosomal damage in the
rodent micronucleus test (TG 474)    living animal
|
All negative: the substance is treated as non genotoxic
Any positive: weight of evidence review and follow up testing
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The Ames test: reverse mutation in bacteria



A dose related rise in revertants, in either arm, is a positive result (TG 471)

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Figure: The Ames test runs every strain and dose with and without the S9 liver fraction, because metabolism creates many mutagens from inactive parent substances.

3.10 In vitro chromosomal aberration and micronucleus tests

The **in vitro chromosomal aberration test (TG 473)** cultures mammalian cells, classically Chinese hamster ovary cells or human lymphocytes, exposes them with and without S9, and scores metaphase chromosomes under the microscope for breaks, exchanges and other structural aberrations. The **in vitro micronucleus test (TG 487)** reads the same class of damage more simply: micronuclei are small extra nuclei formed from chromosome fragments or whole lagging chromosomes that failed to join a daughter nucleus at division, and counting them in divided cells detects both clastogens (chromosome breakers) and aneugens (agents that disturb chromosome number).

3.11 In vivo micronucleus and chromosomal aberration tests

The in vivo arm moves the question into the whole animal, with real absorption, metabolism and DNA repair. The **rodent micronucleus test (TG 474)** uses bone marrow or peripheral blood: immature red blood cells normally expel their nucleus as they mature, and any micronucleus left behind records chromosomal damage suffered in the marrow, counted in thousands of young erythrocytes after dosing. The **in vivo chromosomal aberration test (TG 475)** scores metaphases in bone marrow directly. The in vivo comet assay (TG 489), which measures DNA strand breaks in individual cells by electrophoresis, is a common follow up when a specific organ, such as the liver, needs examination. Positive in vitro results that cannot be reproduced in vivo at adequate exposure are weighed as evidence, not verdicts: the battery is read as a whole.

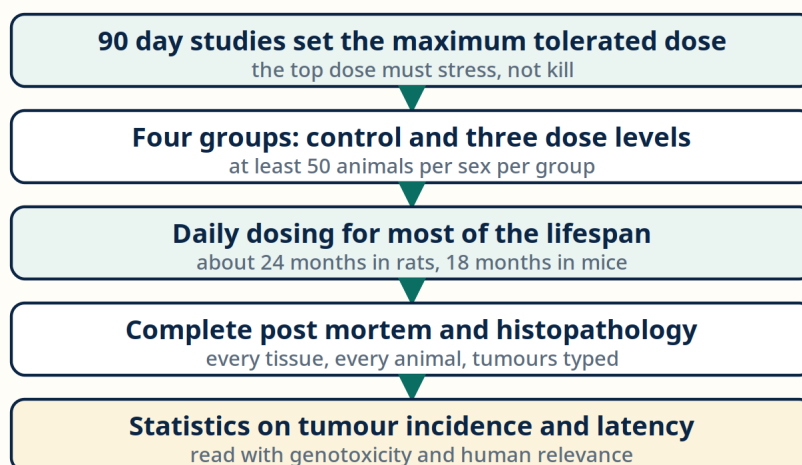
3.12 Carcinogenicity: the long shadow

A **carcinogen** produces tumours. Regulatory thinking divides carcinogens into genotoxic carcinogens, which damage DNA directly and are assumed to have no threshold, and non genotoxic carcinogens, which act through sustained mechanisms such as hormonal disturbance, chronic tissue injury or receptor driven proliferation, and may have thresholds. Unit 3's genotoxicity battery exists to find the first kind early; the in vivo bioassay of the next section exists because the second kind can only be found by long exposure of living animals.

3.13 In vivo carcinogenicity studies: the two year bioassay

The standard **carcinogenicity study (TG 451)** doses rodents, classically the rat and the mouse, at three dose levels plus control, with at least 50 animals per sex per group, for most of the lifespan: about 24 months in rats and 18 months in mice. The top dose is the maximum tolerated dose found in the earlier 90 day studies, high enough to stress the animal without shortening its life by other toxicity, because a study in which the high dose kills early cannot read late tumours. Every animal receives a complete post mortem and histopathology of the full tissue list; tumours are recorded by type, site, malignancy and time of appearance, and statistics compare incidence and latency against concurrent and historical controls. TG 453 combines this design with chronic toxicity satellites to answer both questions in one study. Because the bioassay takes years and hundreds of animals, ICH S1 allows weight of evidence arguments and, increasingly, six month studies in transgenic mouse models (such as rasH2) as part of the assessment for some substances. The result is read with the genotoxicity data: a genotoxic substance with tumours is a clear carcinogen; a non genotoxic substance with tumours only at the maximum tolerated dose invites a mechanistic argument about human relevance.

The in vivo carcinogenicity bioassay (TG 451)



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Figure: The in vivo carcinogenicity bioassay, from the 90 day studies that set the top dose to the statistics that read the tumour outcome.

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