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Hormonal Carcinogenesis

Andrea Terron

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Criteria laid down in EC Regulation 2018/605:

1) *It shows an adverse effect in an intact organism or its progeny, which is a change in the morphology, physiology, growth, development, reproduction or life span of an organism, system or (sub)population that results in an impairment of functional capacity, an impairment of the capacity to compensate for additional stress or an increase in susceptibility to other influences;*

2) *It has an endocrine activity, i.e. it has the potential to alters the function(s) of the endocrine system;*

3) *The substance has an endocrine disrupting mode of action, i.e. there is a biologically plausible link between the adverse effect and the endocrine activity.*

ED definition in a broader context

- From an endocrinological perspective, this definition would be likely considered non-adequate and any endocrine disease linked to environmental chemicals will be enough for the identification of a substance as endocrine disrupters.
- From the toxicological perspective, the definition is also misleading and any substance impacting on the endocrine system would be considered as an "endocrine toxicant" and, as such, assessed in its entire complexity.
- Therefore, this definition has mainly (or uniquely) a regulatory value and specifically refer to hazard identification; the identified hazard should be linked to the endocrine activity as a matter of time and dose concordance.

Hormones and tumours

- The link between hormonal deregulation and occurrence of cancer is well established.
  - Therefore, exploring for hormonal carcinogenesis is a regulatory risk assessment activity.
- Regulatory toxicology lies on standard studies exploring endpoints for hazard identification.
- Is however the carcinogenicity study the right experimental tool to assess hormonal carcinogenesis ?
- Is the carcinogenicity study the best option to define the regulatory implications for chemicals suspected of having an endocrine activity linked to the occurrence of neoplastic diseases ?

10 Key Characteristics of Carcinogens (Smith 2016)

- Electrophilic
- Genotoxic
- Causes genomic instability
- Induces epigenetics alterations
- Induces oxidative stress
- Induces chronic inflammation
- Is immunosuppressive
- **Modulates receptor-mediated effects**
- Causes immortalization
- Alter cell proliferation, cell death or nutrient supply

Key Characteristics of Carcinogens (Smith 2016)

- **Modulates receptor-mediated effects**
  - Intracellular activation, mediated by nucleus receptors that translocate into the nucleus and act on DNA as transcription factor.
  - Activation of cell surface receptors that induce signal-transduction pathways resulting in biological responses.

- **Pathways regulated through ligand-receptor interaction are the most relevant to carcinogenesis and include cell proliferation (e.g. oestrogen-dependent tissue and hormone therapy).**
- **Therefore, chemicals having a link between hormonal perturbation and tumour are likely met one of the key characteristics.**

Is the carcinogenicity study the right tool to assess hormonal carcinogenesis ?

The carcinogenicity paradigm

- Target cells –stem/intermediate cells (progenitors).
- Target genes-oncogenes and tumour suppressors.
- Individual susceptibility-hereditary and exposure to carcinogens.
- Relevant alterations-mutations and epigenetic alteration of target genes.
- Clonal expansion and genetic instability results in an accumulation of mutations with their downstream effect: cancer.

Therefore cancer needs time to develop and any model of cancer is requiring enough time to develop; aging disease (spontaneous and chemically induced).

Data describe the model, not the reality

The link between the carcinogenicity paradigm and the 2-year rodent bioassays is fundamentally based on two basic assumptions:

- Rodent carcinogens are human carcinogens.
- The tumour response at the doses used in the model (MTD) are relevant to human exposure levels (dose extrapolation).

Predicting carcinogenicity

- **What does the bioassay really tell us ?**
  - The human carcinogens that are positive in the bioassays are only few and the presumption that the bioassays can have the capability of predicting potential human carcinogens has no scientific basis.
  - Positive results are most of the time the result of high dose artefact.
  - If a chemical is negative at the MTD is very unlikely that it will cause cancer in human.

Making sense of hormonal carcinogens

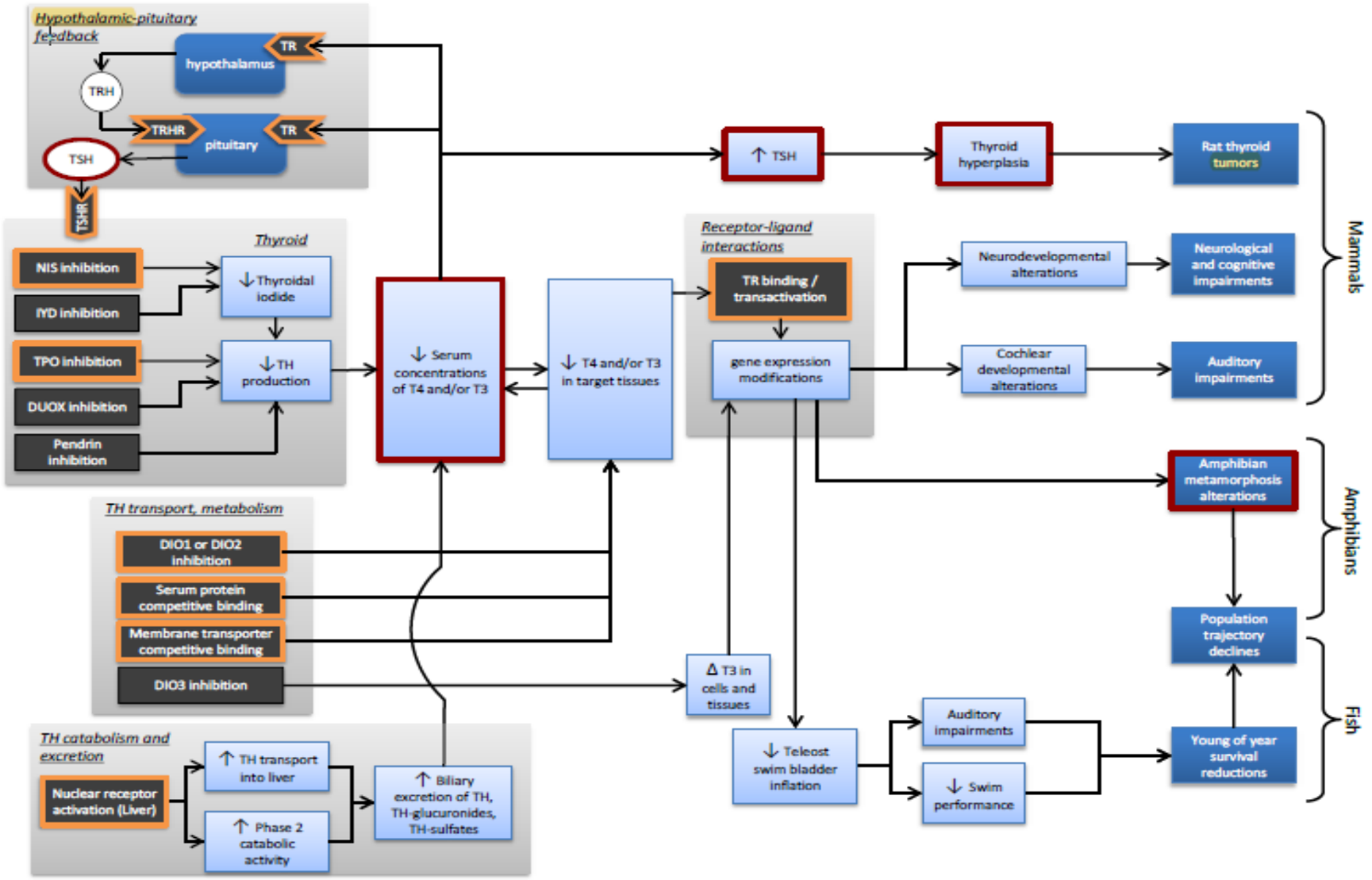
- Considering the intrinsic weakness of carcinogenicity studies, making sense of hormonal carcinogenesis is critical in the current regulation for pesticides and biocides
  - Identify substances fulfilling the criteria for ED.
  - Contextualize human relevance.

Example: thyroid neoplasms

- The hormonal carcinogenesis for the thyroid considers the role of TSH in the development of thyroid tumors.
- Though the role of TSH is critical for the development of thyroid tumors in rodent, only recently the predictive value of TSH, and its association with papillary and follicular thyroid cancers was demonstrated.
  - However the role of TSH as initiator or promoter is still not resolved.
- TSH is a key factor in follicular cell mitogenesis, though a permissive role of insulin and IGF 1 is necessary.

Example: thyroid neoplasm

- The signaling pathway in normal thyrocytes indicates:
  - Genetic changes in human thyroid cancers involve the TSH pathway with activation of elements of de-differentiating signaling pathway.
  - This was also demonstrated in transgenic models, indicating that TSH signaling is a necessary, but not sufficient, condition.
    - The activation of de-differentiating pathways is required for thyroid cancers to develop.
- Following the MoA analysis, chemicals that induce elevated TSH levels in rodents, frequently also induce follicular tumors in rodents.
- This KE is common to many MIEs.


Hypothalamic-pituitary feedback
hypothalamus
TR
TRH
TRHR
pituitary
TR
TSH
TSHR
Thyroid
NIS inhibition
IYD inhibition
TPO inhibition
DUOX inhibition
Pendrin inhibition
↓ Thyroidal iodide
↓ TH production
↓ Serum concentrations of T4 and/or T3
↓ T4 and/or T3 in target tissues
Receptor-ligand interactions
TR binding / transactivation
gene expression modifications
↑ TSH
Thyroid hyperplasia
Rat thyroid tumors
Neurodevelopmental alterations
Neurological and cognitive impairments
Cochlear developmental alterations
Auditory impairments
Mammals
Amphibian metamorphosis alterations
Amphibians
Population trajectory declines
Fish
TH transport, metabolism
DIO1 or DIO2 inhibition
Serum protein competitive binding
Membrane transporter competitive binding
DIO3 inhibition
Δ T3 in cells and tissues
↓ Teleost swim bladder inflation
Auditory impairments
↓ Swim performance
Young of year survival reductions
TH catabolism and excretion
Nuclear receptor activation (Liver)
↑ TH transport into liver
↑ Phase 2 catabolic activity
↑ Biliary excretion of TH, TH-glucuronides, TH-sulfates

Example: thyroid neoplasm

- Rodent thyroid tumors are therefore relevant for the identification of substances having endocrine disrupters properties as the increase in TSH reflects effect on endocrine activity.
- However, the relevance for human cancer risk assessment is often debated because of the quantitative differences of rats and humans (i.e. higher turnover and clearance of TH in rodents).

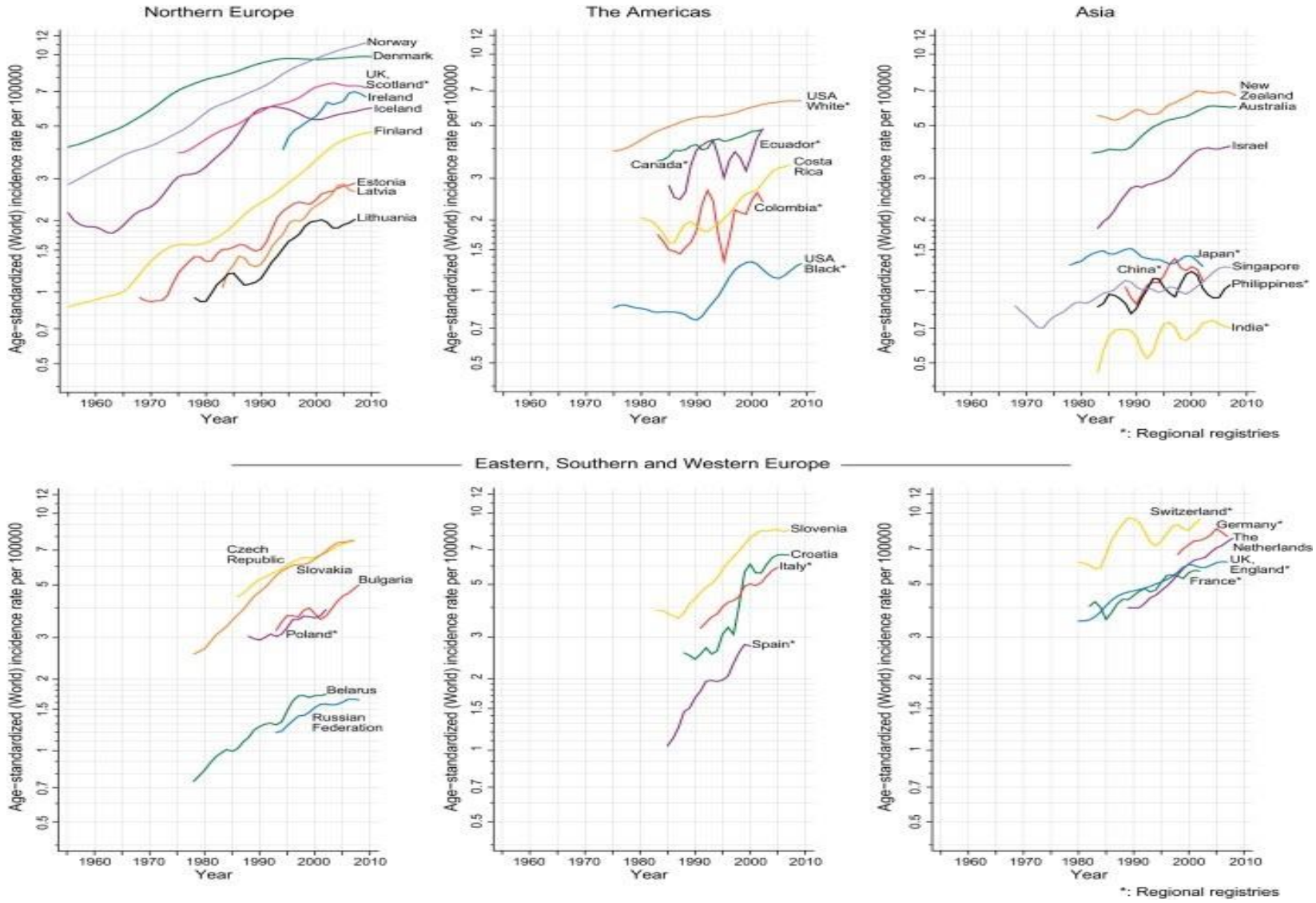
Two faces of the same problem: rat sensitivity

- Carcinogenesis
  - Despite the high TH clearance, mice is much less sensitive to thyroid tumors; this would make the rat sensitivity less relevant.
  - TBG is low also in dog where thyroid effect are rarely observed.
  - With conazoles, tumors are evident without increase of TSH; though, a de-differentiation pathway may play a role.
  - MoA should include transcriptomic and proliferative data to provide a better match with human thyroid cancer by assessing de-differentiation and proliferative pathways.
  - Rodent thyroid cancer may therefore pose a cancer hazard to humans.
  - Chemical specific data should quantitatively explore all the KEs to support non-human relevance.

Two faces of the same problem: rat sensitivity

- Endocrine disruption
  - The MoAs leading to thyroid tumors share KEs that are relevant for endocrine disruption.
  - In this perspective, chemicals that induce thyroid tumors following an endocrine mode of action should be considered to met criteria for ED.
  - Therefore, any quantitative analysis intended to dismiss human relevance should consider the most sensitive population.
  - Dismissing the cancer-related hazard is not enough
  - MoA including liver-induction KEs are relevant for ED and should be specifically addressed.

Testicular neoplasms; human trend



Skakkebaek et al. 2015

Example: Leydig cell neoplasm

- Rat, Leydig (interstitial) cells hyperplasia and adenoma
  - Common in rat after 1 year
  - Adenoma begins as hyperplasia and distinction among the two is based on morphological criteria.
  - Malignancy is very rare.
  - Observed in association with chemicals :
    - GnRH (not human relevant), DA agonists (not human relevant), AR antagonists, 5 alfa reductase antagonists, inhibitors of testosterone synthesis.
- Rat seminoma
  - Rare.

Example: Leydig cell neoplasm

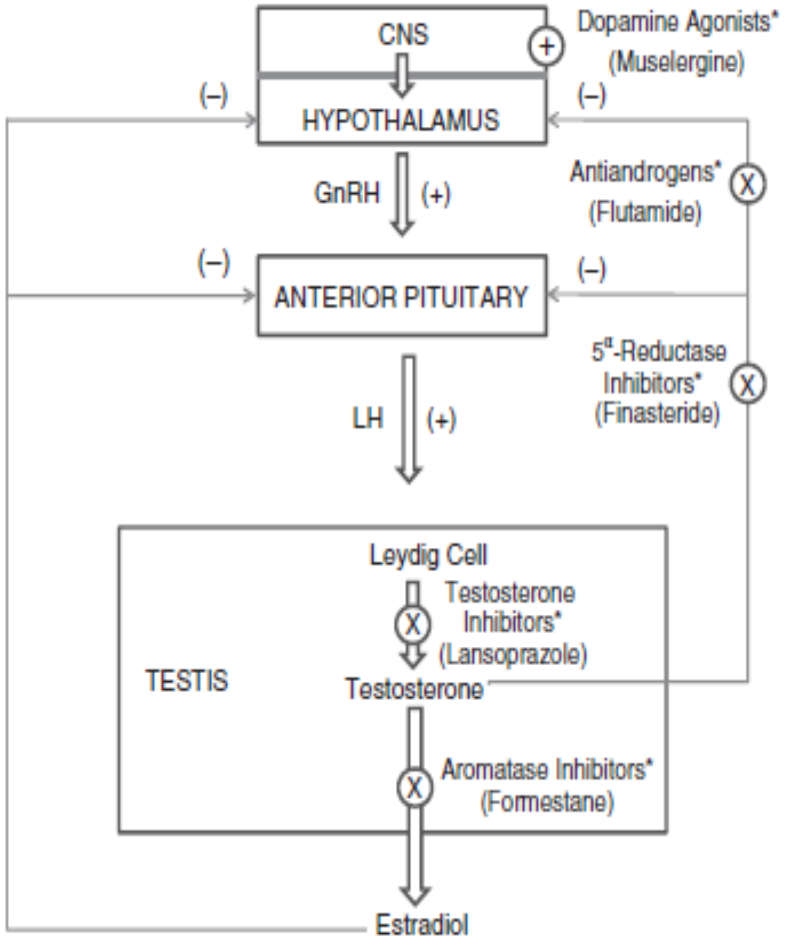
- Human Leydig cell tumor(LCT)
  - Rare (0.4 per million), detection bias to be considered.
  - Through the cancer registries, there is no association between chemical exposure and Leydig cell tumors.
  - Quantitatively, man is considered less sensitive than rats in the proliferative response to LH and consequently to chemically induced LCT.
  - The role of growth factors in LC tumorigenesis remains to be determined.
- Overall considered of no human relevance based on **quantitative** considerations.

Example: Leydig neoplasm

All known potentially human relevant MoAs have impairement of the HPG axis as a common KE.

Changes in LH are unlikely to be enough and proliferative effects should also consider paracrine factors.

Should therefore the LCT be considered as an endpoint indicative of endocrine disruption ?



Is not only a matter of MoA, but also WoE

- LCTs in rodents generally occur in older animals.
- Imbalance between positive and negative regulators occurs with advancing age and, at least in the rat, absence of inhibitory regulators are important.
- The senescence of LC
  - In humans is accompanied by a decrease in testosterone which occurs in the presence of maintenance or increase in circulating LH levels and is the result of a loss in LC.
  - As rat LC age, they likely increase in number without an increase in LH or without an increase in LH stimulation (sometime decline) with a concomitant decline in the ability of producing testosterone.
    - At senescence, chemicals that perturb the HPG are likely resulting in a decrease latency of senescence LC.

Example: Leydig cell neoplasm

- The adult LC
  - In the absence of cytotoxicity or damage to the seminiferous epithelium, LC are not very responsive during the adult phase to perturbation of LH and different endpoints are more sensitive.
- The pubertal LC
  - In both man and rat, LH and androgens are involved in the morphological and functional differentiation of LC precursors. At this stage, in both species, FSH is also important in the regulation of development of the adult LC.
  - Testing for hormonal changes and endocrine sensitive endpoints can help in understanding if the HPG axis is deregulated and if a pattern of endocrine disruption exist in the most relevant population.

Example: Leydig cell neoplasm

- In rodents, LC hyperplasia and adenoma are likely representing exacerbation of the physiological senescence.
- In human, the dominant morphological effect is rather associated with LC atrophy.
- To translate this adverse outcome in a pattern of effects indicative of endocrine disruption the overall weight of evidence should focus on the status of the HPG axis.
- In a complete dataset, the most vulnerable population dealing with perturbation of the HPG axis is likely represented by the peripubertal age.
- Hormonal assessment should be therefore carefully assessed across the dataset and sensitive endpoints at peripubertal age should be assessed.

Conclusions

- There is a biological plausible link between hormonal deregulation and tumor development.
- Hormonal mediated effect is a recognized key characteristic of carcinogens.
- Carcinogenic studies are intended to explore the carcinogenic potential of a chemical.
- Using the hormonal carcinogenesis to identify (predict) ED substances is however complex and should be case by case.
- The overall WoE to come to this conclusion should consider the full and complete dataset.
- Once the MoA is established, key studies should focus on the most sensitive population for the definition of ED properties.

Few references

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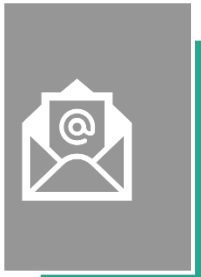
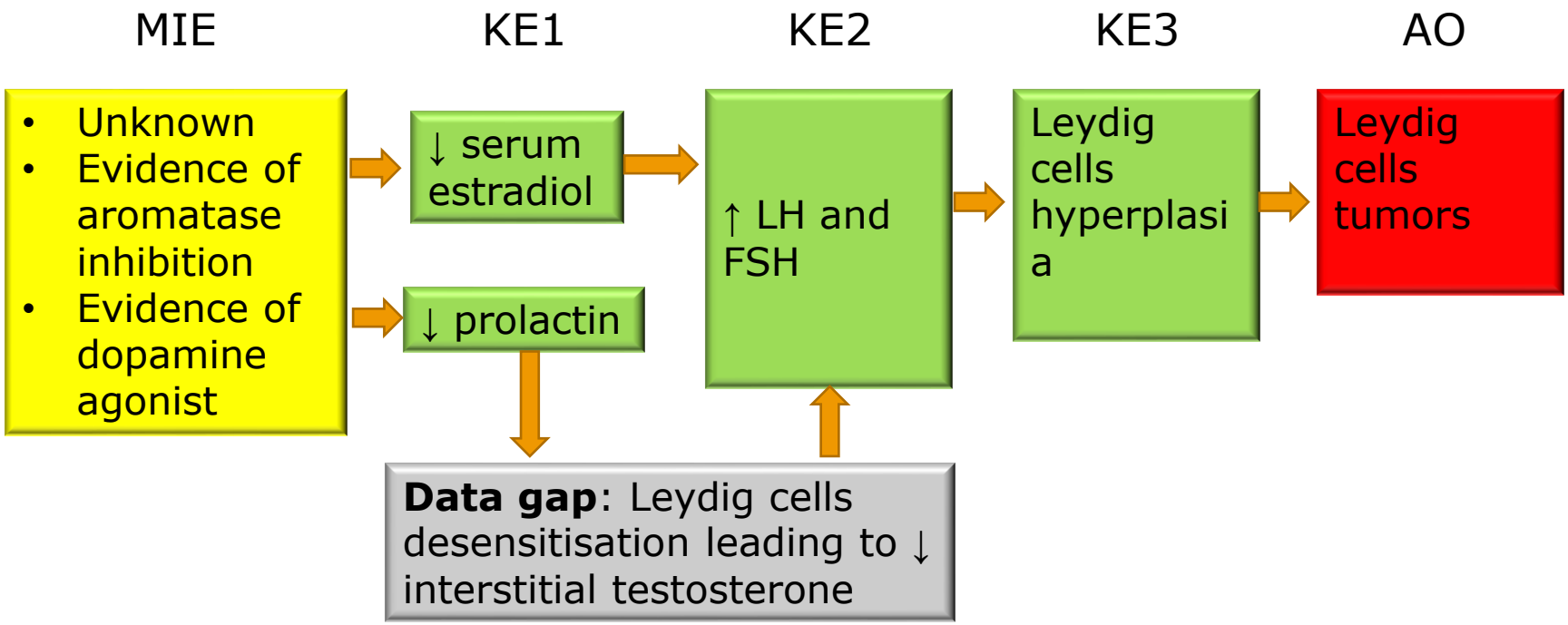


Email

ask@efsa.europa.eu

Example: Leydig cell neoplasm



Example: Leydig cell neoplasm