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View of a developmental toxicologist from the EU on the Japanese proposal

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The Japanese proposal,
presented by Prof. Michio Fujiwara

pivots on the update of definitions and re-categorisation of grey zone findings

Why bothering about gey zone findings?

Findings that are currently difficult to categorize either as variations or malformations
Are frequently observed in Prenatal tox studies

In the EU, their interpretation is relevant to the regulatory outcome of a substance
as it can influence the classification under cat.1, cat. 2, or no classification
For developmental toxicity

E.g., under the REACH cat. 1 as developmental toxicant means "*Substance of Very High Concern*" with heavy regulatory consequences

Thus, understanding whether "gray zone anomales" may represent, indicate or predict *health-relevant* developmental effects is definitely *worthwhile*

***Updated OECD 414* (2018) requires measurement of anogenital distance (AGD)**
Reduced AGD in males, without a reduction in weight,
***Per se* not a malformation, rather a grey-zone**
But indicates intrauterine endocrine disruption
And predicts a reproductive impairiment in later life

In this case the relevance of the grey-zone change relies on the *understanding of the underlying biology*

The JTS on grey zone reduction deserves merit
a) is an effort to reduce uncertainties in the interpretation of data
b) the effort is based on biological considerations,
i.e., the likely origin of the anomaly and its likely consequence(s)

Functional changes: *observable effects* (e.g., discoloration, hemorrhages) that reflect an input after organogenesis
Should be considered *as adverse*: indicate pharmaco-toxicological effects

Permanent structural changes: represent an input during organogenesis
Adversity? *unknown* effect on health or survival
Albeit it is *precautionary* to consider them adverse.

The two clusters are correctly identified as two distinct components

1) Functional changes: express adversity after morphogenesis,
Different from growth retardation (fetal weight) and fetal death
***AGD* may be considered as related,**
but is an *objective measurement* (continuous parameter)
Opposite to *qualitative* (discrete parameters)
- Further consideration to introduce *other measurements ??* (length of skeletal segments??, organ size ??)

AGD is clearly related to an AOP
- Other "functional" changes are obviously adverse and may be -mainly or somewhat – related to Relevant modes of action (dose-relationship and plausibility linking to treatment)
- In other instances adversity is suspected (cannot be excluded)

1) Functional changes: discussion on examples provided

Foetal oedema: clearly adverse, associated with perinatal death

- (in the absence of cardiovascular or poly-malformations) may result from:
autommunity; placental dysfunction; functional damage to cardiovascular or lymphatic systems
(for human *hydrops fetalis* see Bellini et al., Am J. Med Genet, 2015)

Fetal hemorrhages (external; internal may make a part of organ “discoloration”)
may result from toxicological or adverse pharmacodynamic effects on fetal endothelium or blood pressure (e.g., dose-related increase of external hemorrhages with ammonium glycyrrhizinate, Mantovani et al., Food Chem Toxicol 1988)

1) Functional changes: discussion on examples provided

Bladder distension has been extensively studied and coded in human medicine:
Non-syndromic cases may reflect
defective functional development of renal cortex,
of the urthral-ureteral system
or an altered exchange with embryonic adnexa (amnios)
(see e.g., Montemarano et al., J Ultrasound Med. 1998; Nguyen et al., J Pediatr Urol. 2014)

Other instances of "distension" and "discoloration" are less clear or remain unexplained.
However -unless a different explanation is available-
it is sound to consider these changes as
indicator/predictors of potentially adverse functional effects

Since some main components of this group are at the border between functional and structural (e.g., edema, hemorrhages)

Rather than “non-structural functional change”

It is suggested to adopt this new group with the name Functional changes

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2) Permanent structural changes:

Adverse consequences are still unknown, *but*
Clearly indicate that *site/tissue/organ specific* morphogenesis is *affected*
It is *both conservative and scientifically sound* to group with malformations
while extending the *definition* of “malformation”

Among the proposed components of this cluster
Some are *already identified as malformations*, because they are associated with impaired functions

Fusion of skeletal structures
Clearly impair the movement and flexibility of the trunk (fused vertebrae, sternebrae)
Action of limbs (fusion of phalanges)
Impaired brain growth (fusion of cranial bones)

2) Permanent structural changes:

- **Malposition of vessels**

Although they may be present in isolation
They are part of recognized cardiovascular dymorphogenesis complexes (Fallot's tetralogy etc.)
In humans they have variable severity, but they are also a component of clinically relevant anomalies
(Bravo-Valenzuela et al., J Matern Fetal Neonatal Med. 2019)

- consider also other maòlpositions such as
Renal displacement
(rat kidneys at the same height: right should be higher):
may indicate an improper development of the architecture of the excretory system

2) Permanent structural changes:

- Absent or supernumerary lobes in organs like liver and lung (Absent skin like other absent structures will be clearly related to evident adversity)

- Lung: adversity not clearly evident, however *No information on histopathology* (normal? Abnormal?)

- In humans they reflect abnormalities in tracheobronchial branching, which occur early in organogenesis (month 1)

- Often asymptomatic, but *may increase the risk* of pneumonia and dyspnoea later in life (see e.g., Chassagnon, Radiographics 2016)

- Liver: adversity not clearly evident,

In humans *lobar anomalies* (mainly absence of the causate lobe) is reported:

- patients have a higher frequency of biliary disturbances or portal huypertension (see, e.g., Radin et al., Radiology, 1987)

- Possibly, lobar anomalies flag an altered internal architecture

MALFORMATION

Current Definition

A permanent structural change that is likely to adversely affect the survival or health of the species under investigation. (Reproductive Toxicology 13, 77-82, 1999)

Additional definition **(proposal by JTS)**

A permanent structural change resulting from an abnormal developmental process but without evidence or information about the adverse effect.

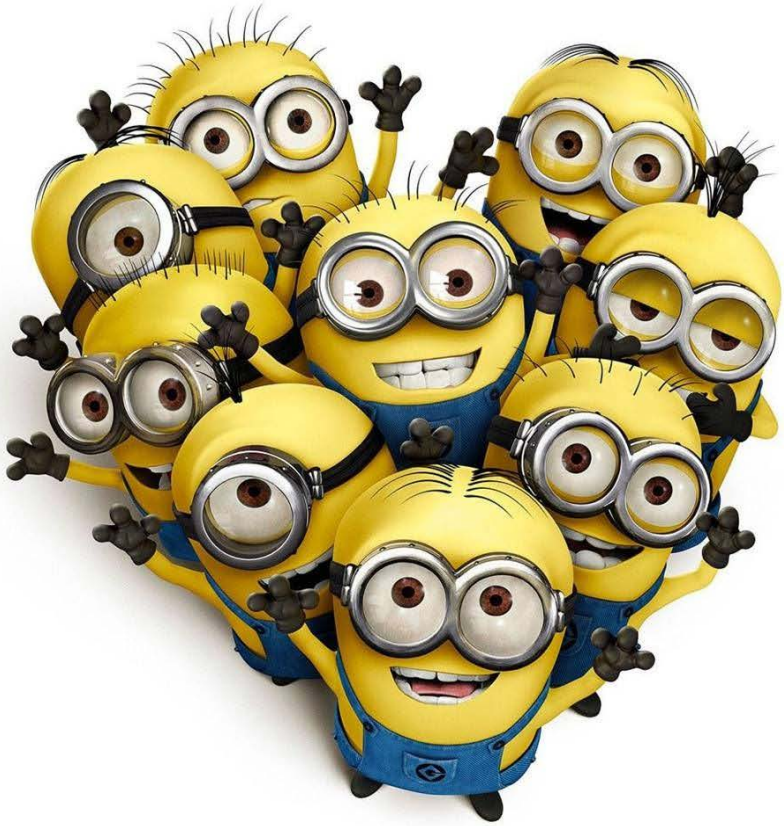
Attempt to integrate

A permanent structural change resulting from an abnormal developmental process that can adversely affect, or is plausibly linked to an adverse effect on, the survival or health,

POSSIBLE STEPS FORWARD

Use of computerized image analysis in order to introduce *quantitative parameters*
“enlarged” “small” “dilated”
There is a threshold? (based on concurrent controls)

Use of an AOP-like (Adverse Outcome Pathways) approac to link morphological anomalies to embryological events (upstream) and adversity (downstream)
While AOP are mainly concentrated on molecular and sub-cellular events to be linked to adversity
In this field, main focus should be to organogenetic events at tissue level



THANK YOU!

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