Berlin Workshop view on a new survey for recategorisation of grey zone anomalies

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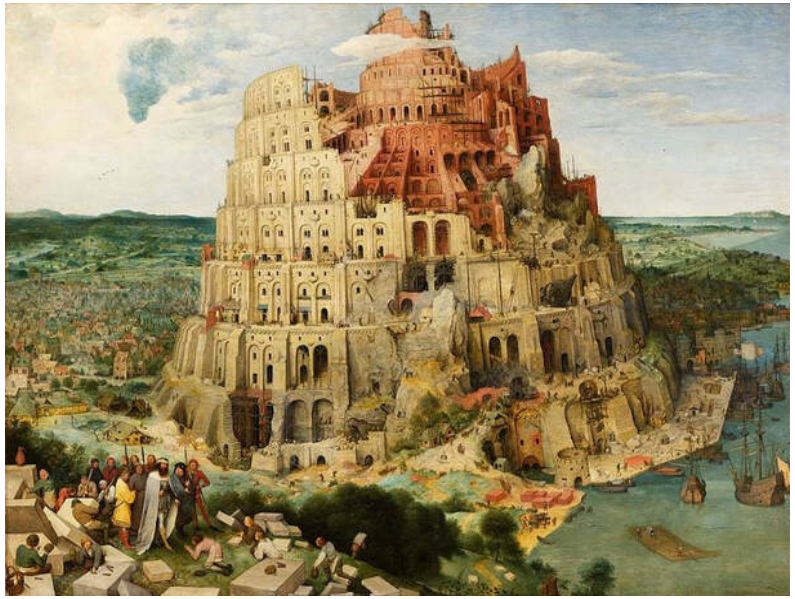
Background

On the revision of the nomenclature for structural abnormalities

- *Lack of a common vocabulary to communicate scientific findings may lead to misinterpretation, confusion and uncertainty. This is an even greater problem when data from toxicity studies are used for health risk assessment and regulatory decision-making.*

- *It is consensual that a common glossary of terms must be provided and used to describe fetal observations in DevTox studies.*

- *With this in mind, a first version of an internationally-developed glossary of descriptive terms for structural developmental abnormalities in common laboratory animals was the main goal of the 1995 inaugural workshop of this series of Berlin DevTox Workshops.* *(Wise et al, Teratology 1997,55:249-292)*



Pieter Bruegel the Elder's *Tower of Babel* (1563)

A common internationally-agreed vocabulary is necessary to speak the same language when reporting DevTox study findings.

Background

On the harmonization of the classificatory terminology

- *Ambiguities and inconsistencies in the use of terms to classify structural anomalies in Dev Tox study reports is also a major problem, particularly for health risk assessors and administrative decision makers.*

- ***To classify or not** (Is there a need for classification?) and the harmonization of the classificatory terminology used for fetal observations were the main topics of discussions held at the second Berlin WS in 1998.*

Second Berlin Workshop – 1998

- *Although there was a strong opinion against classification of findings, a majority agreed that – for practical reasons - it is needed for regulatory decisions and chemical labelling schemes.*
- *A classification scheme for fetal structural abnormalities consisting of only two categories (malformation or variation) was then advanced.*

(Chahoud et al, Reproductive Toxicology 1999, 13:77-92)

Classification scheme (*2nd Berlin Workshop, 1998*)

***Malformation* (M)**:

"*a permanent structural change that is likely to adversely affect the survival or health of the species under investigation*"

***Variation* (V)**:

"*a change that occurs within the normal population under investigation and is unlikely to adversely affect survival or health*"

***Undetermined* (U)**: *"cannot decide between M or V"*

***Not known* (N):**

"Gray area / zone" *a group of observations that do not readily fit into the* ***M*** *or* ***V*** *category*

Exercise to apply the categorization to terms listed in the IFTS Glossary (V-1) (skeletal anomalies) by attendees of the 2nd Berlin workshop:

Table 2. Examples of malformations and variations of a rat foetus at term according to the classification scheme discussed at the Workshop

Skeletal abnormality	Code No.	M	V	U	N
Malpositioned metacarpal	10597	X			
Misshapenned metacarpal	10598	X			
Small metacarpal	10599		X		
Supernumerary metacarpal	10600	X			
Unossified metacarpal	10601		X		
Absent phalanx	10602	X			
Fused phalanx	10603	X			
Incomplete ossification of phalanx	10604		X		
Malpositioned phalanx	10605	X			
Misshapenned phalanx	10606	X			
Small phalanx	10607	X			
Supernumerary phalanx	10608	X			
Thickened phalanx	10609		X		
Unossified phalanx	10610		X		
Absent sternebra	10611	X			
Bipartite ossification of sternebra	10612		X		
Extra sternebral ossification site	10613		X		
Fused sternebra	10614			X	
Incomplete ossification of sternebra	10615		X		
Malpositioned sternebra	10616	X			
Misaligned sternebra	10617			X	
Misshapenned sternebra	10618			X	
Sternochisis	10619	X			
Unossified sternebra	10620		X		

The participants were asked to classify either as a malformation or as a variation the skeletal abnormalities (IFTS terminology) listed below. M = malformation; V = variation; U = can't decide between 'M' and 'V'; and N = term not known/not used in the laboratory. Only in three cases (U) was no consensus reached among participants.

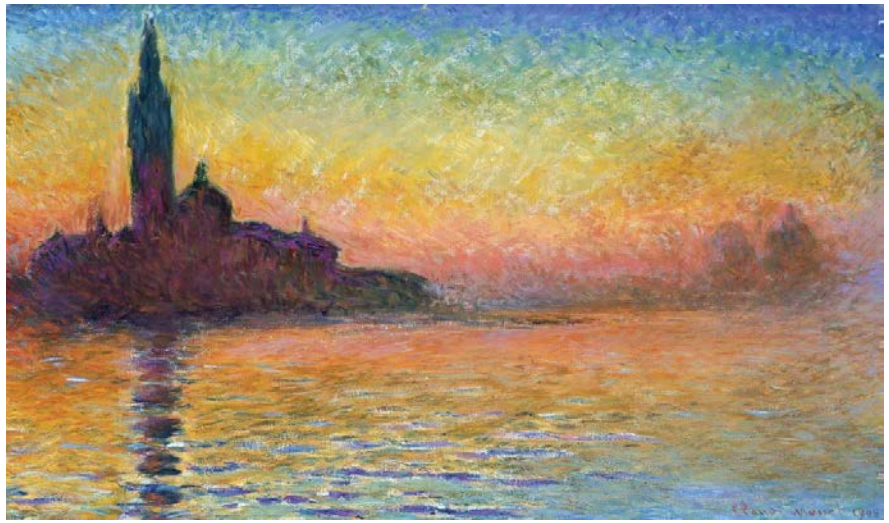
No consensus

(Chahoud et al, Reproductive Toxicology 1999, 13:77-92)

Grey zone anomaly ► *An actual or potential fetal observation that is not clearly a malformation (**M**) or a variation (**V**).*

*The term **"Grey zone anomalies"** refers to a group of (potential) fetal observations which experts who took part in the BW surveys could not make a collective decision on whether they should be **M** or **V.***

***Grey zone** anomalies are, therefore, a **group of actual or potential observations**, **not a** (third) separate **category** of fetal observations.*



Twilight in Venice – Claude Monet, 1908

Grey zone

- *Area of uncertainty or indeterminacy*
- *Intermediate area between opposing positions*
- *Not clearly one thing or the other*
- *Not covered by an existing category or set of rules*

*A number of potential observations (descriptive terms) listed in the Glossary (V.1) (IFTS, 1997) did not readily fit into either (M or V) category of the classification scheme (2nd BW, 1998) as revealed by surveys among experts on categorization of skeletal (2001), external and visceral observations (2003). Based on the survey results, these observations were grouped as **grey zone** anomalies.*

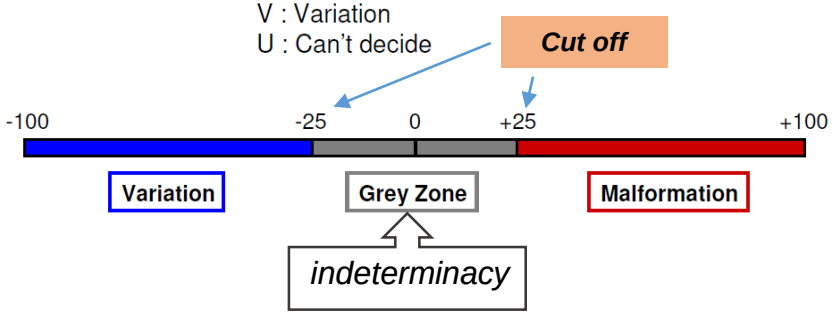
Categorization based on survey results

Not necessarily achieving a consensus, but a substantial agreement among evaluators

- Index of Agreement (IA):

$$IA = [(M-V) \div (M + V + U)] \times 100$$

M : Malformation
V : Variation
U : Can't decide



Solecki et al, Reprod Toxicol 2001, 15:713-721

Solecki et al, Reprod Toxicol 2003, 17:625-637

Malformation | Grey zone | Variation

Table 4
Indices of agreement for sternebrae and vertebrae

Bone	Absent	Fused	Malpositioned	Misaligned	Misshapened	Incomplete ossification	Unossified	Small	Hemivertebra	Supernumerary	Hemicentric	Split cartilage	Unilateral cartilage	Bipartite ossification	Dumbbell
Sternebra	84.6	15.4	76.9	−50.0	−15.4	−84.6	−84.6			23.1				−66.7	
Vertebra	100														
Cervical arch	100	92.3	100	−8.3	61.5	−84.6	−53.8	58.3		61.5					
Cervical Ct.	100	92.3		8.3	7.7	−84.6	−46.2			69.2	27.3	23.1	41.7	−53.8	−58.3
Cerv. Ct. Cart.		75.0													−58.3
Cervical Vert.	100		83.3						83.3	46.2					
Thoracic arch	100	84.6	75.0	7.7	15.4	−84.6	−46.2	38.5		61.5					
Thoracic Ct.	100	84.6		8.3	15.4	−84.6	−53.8			61.5	16.7	23.1	33.3	−53.8	−58.3
Thor. Ct. Cart.		69.2													−63.6
Thoracic Vert.	100		66.7						83.3	23.1					
Lumbar arch	100	84.6	83.3	0.0	15.4	−84.6	−46.2	30.8		61.5					
Lumbar Ct.	100	84.6		0.0	0.0	−84.6	−46.2			61.5	36.4	23.1	23.1	−53.8	−58.3
Lumb. Ct. Cart.		83.3													−58.3
Lumbar Vert.	100		83.3						83.3	23.1					
Sacral arch	100	100.0	83.3	−8.3	15.4	−84.6	−46.2	41.7		61.5					
Sacral Ct.	100	84.6		0.0	0.0	−84.6	−46.2			61.5	16.7	33.3	23.1	−53.8	−58.3
Sac. Ct. Cart.		69.2													−58.3
Sacral Vert.	100		83.3						66.7	23.1					
Caudal arch	58.3	41.7	50.0	10.0	18.2	−83.3	−45.5	36.4			0.0			−54.5	
Caudal Ct.	83.3	36.4		0.0	0.0	−81.8	−45.5								−54.5
Caudal Vert.	33.3		36.4						33.3	−36.4					

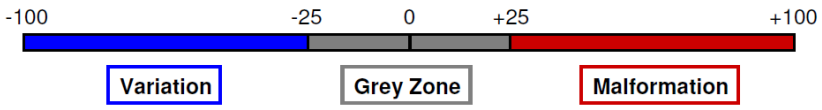
Ct, centrum; Cerv, cervical; Vert, vertebra; Thor, thoracic; Cart, cartilage; Sac, Sacral.

Outcome of the survey among experts on the categorization of skeletal observations (2001).Skull bone findings

- Index of Agreement (IA):

$$IA = [(M-V) \div (M + V + U)] \times 100$$

M : Malformation
V : Variation
U : Can't decide



Malformation **Grey zone** **Variation**

Table 6
Indices of agreement for cranial bones

Bone	Absent	Fused	Small	Misshapened	Split	Unossified	Hole	Incomplete ossification	Bipartite	Bent
Alisphenoid	100	83.3	66.7	83.3		−11.1	−10.0	−55.6		
Auditory oss.	100	100		100		−33.3				
Basioccipital	100	84.6	53.8	69.2		−30.8	−27.3	−84.6		
Basisphenoid	100	100	66.7	83.3		−25.0	−10.0	−83.3		
Exoccipital	100	100	53.8	69.2		−30.8	−25.0	−84.6		
Frontal	100	50.0	53.8	83.3		−16.7	−18.2	−83.3		
Hyoid	100		33.3	23.1		−63.6		−100		−23.1
Interparietal	100	69.2	53.8	76.9		−41.7	−18.2	−84.6	−76.9	
Lacrimal	100	92.3	40.0	70.0		−40.0		−80.0		
Mandible	100	92.3	76.9	84.6				−84.6		
Maxilla	100	100	76.9	84.6		−7.7		−84.6		
Nasal	100	80.0	69.2	84.6		−7.7	−20.0	−76.9		
Palatine	100	100	61.5	84.6	100	−46.2		−69.2		
Parietal	100	100	53.8	84.6		−15.4	−33.3	−84.6		
Premaxilla	100	100	75.0	83.3		−16.7	−20.0	−83.3		
Presphenoid	100	100	55.6	83.3		−50.0	−71.4	−77.8		
Squamosal	100	100	66.7	69.2		−38.5	−83.3	−84.6		
Supraoccipital	100	100	66.7	83.3		−25.0	−30.0	−83.3	−75.0	
Tympanic ann	100	100	41.7	83.3		−50.0		−83.3		
Vomer	100	100	41.7	75.0		−33.3		−77.8		
Zygomatic	100	100	38.5	84.6		−46.2		−84.6		

Solecki et al, Reprod Toxicol 2001, 15:713-721

BW view on the possible reasons for grey zone (GZ) anomalies

***GZ** anomalies in **BW surveys** on the classification of fetal observations (1st version of the terminology glossary)*:

- *Descriptive terms for which there was a **low agreement among evaluators***
- *Descriptive terms for actual or potential observations that **evaluators agreed that do not fit neatly into either category** (M or V).*

*According to BWs' attendees the main **reasons for lower agreement** among evaluators were*:

- ***Imprecise descriptive terms***
- ***Insufficient knowledge on the postnatal consequences***
- *Theoretical terms that are unlikely to occur in isolation*
- *Possibility of observing a range of severity that might be decisive for classification*

Report of the third Workshop on the terminology in DevTox. Berlin,16.09.2000

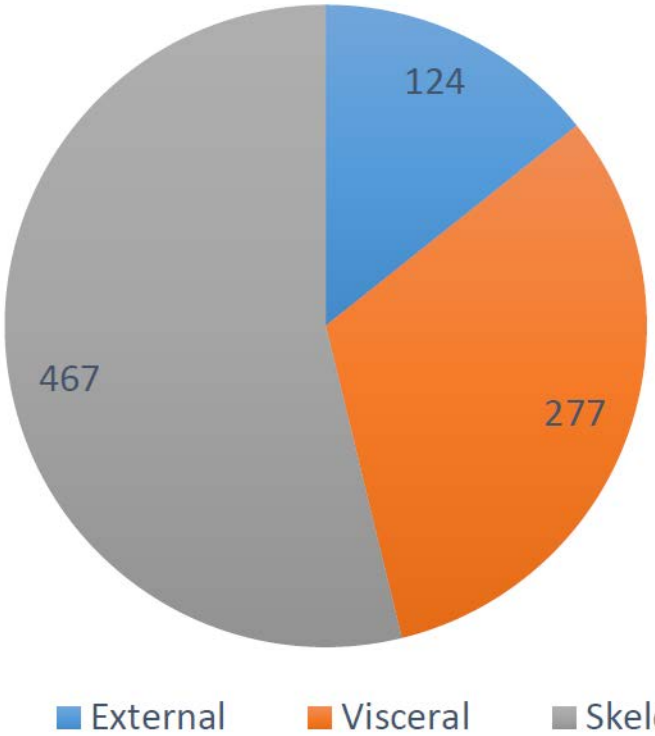
Solecki et al. Reproductive Toxicology 2001, 15: 713-721

1997

TERATOLOGY 55:249–292 (1997)

Terminology of Developmental Abnormalities in Common Laboratory Mammals (Version 1)

L. DAVID WISE,[1,19,20,21]* SIDNEY L. BECK,[2,22] DIANA BELTRAME,[3,19]† BRUCE K. BEYER,[4,22] IBRAHIM CHAHOUD,[5,19] ROBERT L. CLARK,[6,20] RUTH CLARK,[7,19]†† ALICE M. DRUGA,[8,19] MAUREEN H. FEUSTON,[9,20] PIERRE GUITTIN,[10,19]††† SUSAN M. HENWOOD,[11,22] CAROLE A. KIMMEL,[12,19] PIA LINDSTROM,[13,21,23] ANTHONY K. PALMER,[14,19] JUDITH A. PETRERE,[15,22] HOWARD M. SOLOMON,[16,20,21] MINEO YASUDA,[17,19] AND RAYMOND G. YORK[18,20]



N = 868

Update of the glossary and making descriptive terms better

2009

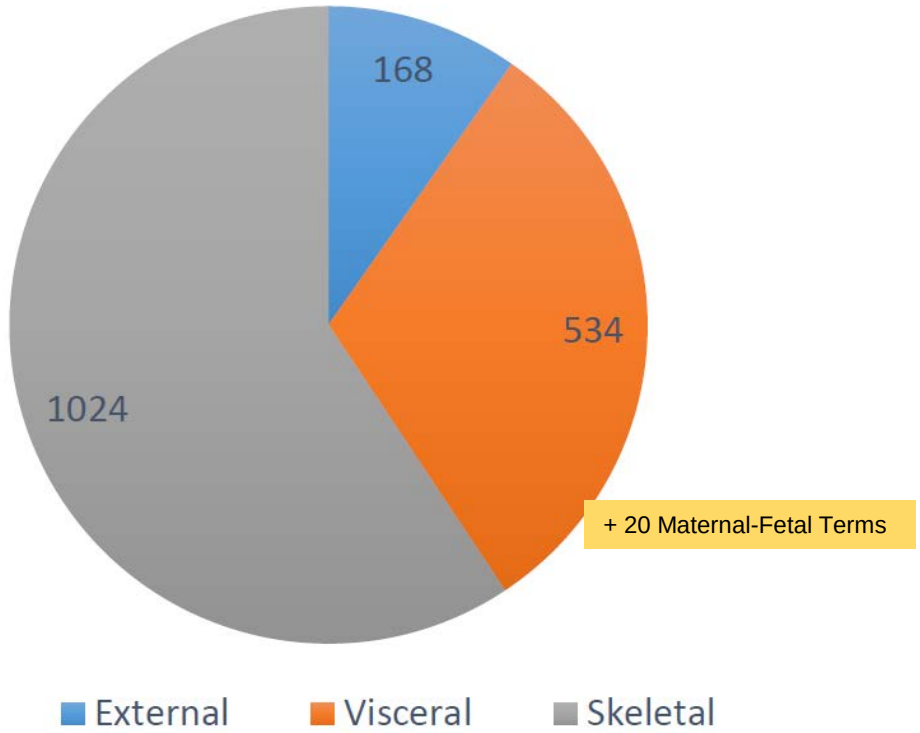
Contents lists available at ScienceDirect

Reproductive Toxicology

journal homepage: www.elsevier.com/locate/reprotox

Terminology of developmental abnormalities in common laboratory mammals (version 2)☆,☆☆

Susan L. Makris[a,*,1,4], Howard M. Solomon[b,1,4], Ruth Clark[c,2,4], Kohei Shiota[d,3,4], Stephane Barbellion[e,2], Jochen Buschmann[f,2], Makoto Ema[g,3], Michio Fujiwara[h,3], Konstanze Grote[i,2], Keith P. Hazelden[j,1], Kok Wah Hew[k,1], Masao Horimoto[l,3], Yojiro Ooshima[m,3], Meg Parkinson[n,2], L. David Wise[o,1]



+ 20 Maternal-Fetal Terms

N = 1726

BW survey of **new descriptive terms** in Version 2 glossary (2013)

Table 1
Categorization of selected external anomalies.

Code used in Version 2	Observation	Index of agreement (%)	Categorization
1.1244.5088	Anus—Large	14	Grey zone
1.1224.5088	Fetus or pup/neonate—Large	−7	Grey zone
1.1246.5088	Genital tubercle—Large	21	Grey zone
1.1235.5088	Snout—Large	53	Grey zone/Malformation
1.1049.5211	Digit—Small	50	Grey zone/Malformation
1.1224.5211	Fetus or pup/neonate—Small	−21	Grey zone
1.1238.5211	Tooth—Small	14	Grey zone

Table 2
Categorization of selected skeletal anomalies.

Code used in Version 2	Observation	Index of agreement (%)	Categorization
2.1002.5250	Alisphenoid—Unossified area	−93	Variation
2.1140.5246	Atlas, ventral arch—Unossified	−64	Grey zone/Variation
2.1140.5253	Atlas, ventral arch—Unilateral ossification	−59	Grey zone/Variation
2.1008.5250	Basioccipital—Unossified area	−88	Variation
2.1008.5252	Basioccipital—Unossified line	−93	Variation
2.1069.5250	Mandible—Unossified area	−88	Variation

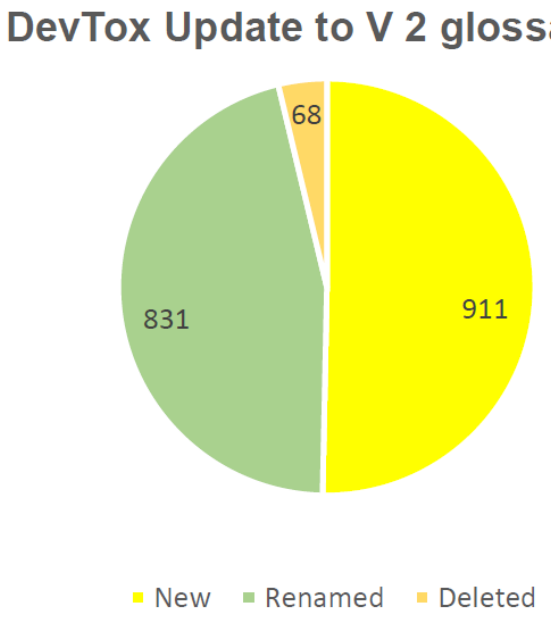
Table 5
Categorization of selected soft tissue anomalies.

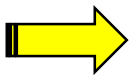
Code used in Version 2	Observation	Index of agreement (%)	Categorization
3.1190.5307	Carotid artery—Branching variation	−100	Variation
3.1082.5307	Posterior (caudal) vena cava—Branching variation	−73	Variation
3.1102.5307	Subclavian artery—Branching variation	−73	Variation

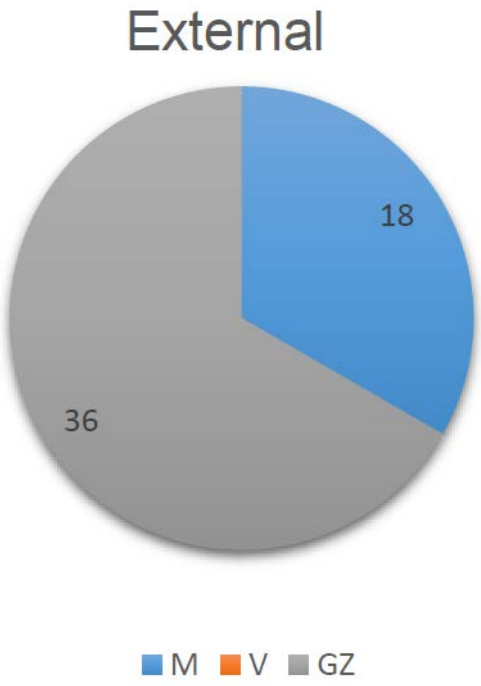
Solecki et al Reprod Toxicol 2015; 57:140-146.

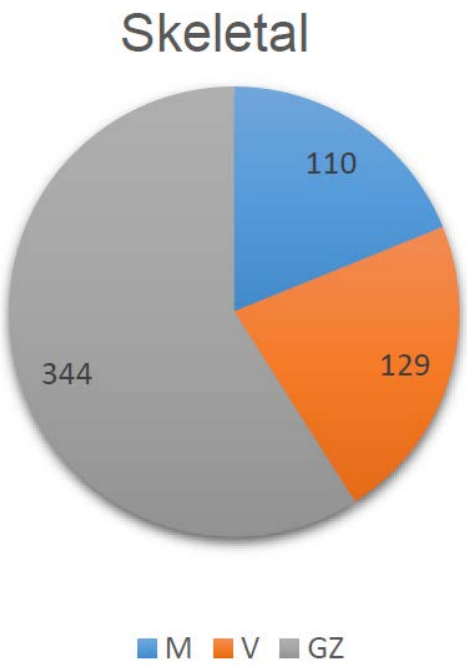
BW survey on the categorization of **new descriptive terms** in Version 2 glossary (2013)

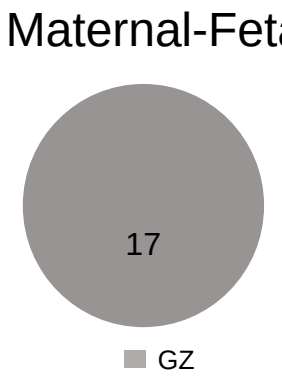
Most V2 new terms fell into Grey zone group

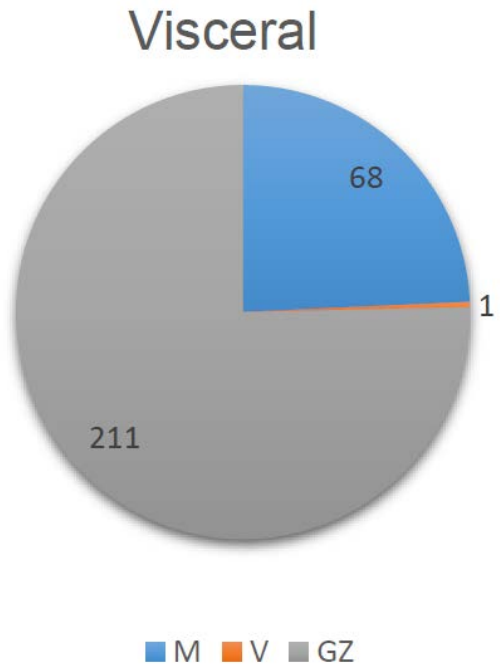












Solecki et al Reprod Toxicol 2015; 57:140-146.

Congenital Anomalies
Clinical and Laboratory Research
THE JAPANESE TERATOLOGY SOCIETY

82 *Congenital Anomalies* 2018; **58**, 82–86 doi: 10.1111/cga.12272

CONSENSUS REPORT

Categorization of fetal external findings in developmental toxicology studies by the Terminology Committee of the Japanese Teratology Society

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Categorization principles of fetal external findings in common laboratory animals were discussed at the Workshop in the 55th JTS Annual Meeting in 2015. As a result, 42 out of 73 Gray zone findings selected were classified as Malformations (38), Non-structural abnormalities (3), "Malformations/Non-structural abnormalities" (1), and no variations (0). The remaining 31 findings were not categorized and left as Not applicable because of various reasons. The details of the classification are shown on the JTS website (http://www.umin.ac.jp/cadb/External.pdf). Our proposal is a scientific recommendation for practical categorization of fetal external findings and is not a requirement; case-by-case judgments based on criteria of each individual laboratory should be accepted.

JTS's proposal to redefine categories

Abnormality:

►"*malformation (structural abnormality)*"

►"*non-structural abnormality*"

OR

Variation:

"*deviation from normal morphology but considered transient or observed frequently in specific species or strains*"

Depends on severity:

"*either abnormality, variation, or neither of them (normal) depends on severity*"

Not applicable:

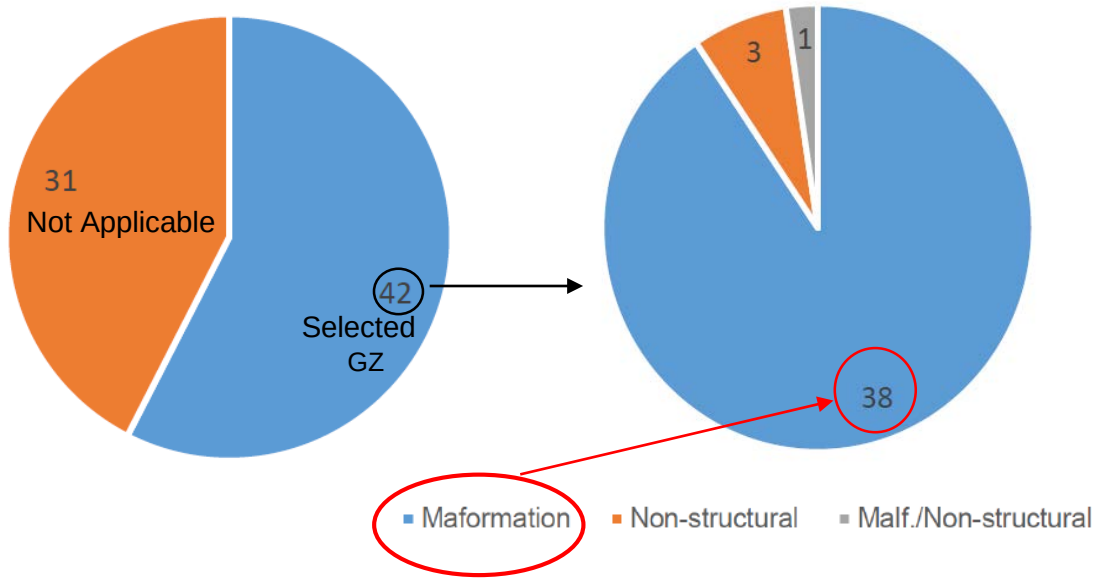
"*unable to classify as abnormality or variation in fetuses*"

Table 1 List of "Abnormality" based on the survey (28 findings: more than 18 facilities or teratology experts selected Abnormality or "Abnormality according to criteria")

Region/organ/structure	Observation		Code no.†	Survey result 1/2/3/4‡	Subclassification
General	Subcutaneous edema	Generalized	10001	22/0/0/0	Malformation or Non-structural abnormality ?
	Skin	Absent	10003	22/0/0/0	Malformation
Head/neck	Head	Domed	10012	22/0/0/0	Malformation
Ear	Pinna	Large	10020	19/1/2/0	Malformation
		Malpositioned	10021	18/1/1/2	Malformation
		Misshapen	10023	20/1/1/0	Malformation
Face	Snout	Large	New	16/0/2/4	Malformation
		Misshapen	10041	20/1/0/1	Malformation
	Tongue	Large	10054	18/1/1/2	Malformation
		Protruding	10065	19/1/0/2	Malformation
Limb	Limb	Hyperextension	10069	18/2/0/2	Malformation
		Hyperflexion	10070	21/1/0/0	Malformation
Paw/digit	Digit	Large	10081	20/0/2/0	Malformation
		Misshapen	10085	20/0/2/0	Malformation
		Small	10079	20/0/2/0	Malformation
	Paw	Hyperextension	10086	18/2/0/2	Malformation
		Hyperflexion	10087	19/2/0/1	Malformation
Tail	Tail	Bent	10094	22/0/0/0	Malformation
		Blunt-tipped	10095	17/0/1/4	Malformation
		Curled	10096	22/0/0/0	Malformation
		Hooked	10099	22/0/0/0	Malformation
		Kinked	10100	21/0/1/0	Malformation
		Misshapen	New	20/0/1/1	Malformation
		Narrow	10102	20/0/1/1	Malformation
Trunk	Genital tubercle	Large	New	15/1/3/3	Malformation
		Misshapen	New	18/1/1/2	Malformation
		Small	10119	17/1/2/2	Malformation
	Trunk	Small	10117	19/0/1/2	Malformation

†New: These findings were added in the revised edition (Version 2) (Makris et al. 2009a,b,c), and thus not numbered.
‡Survey results: 1, Abnormality; 2, Variation; 3, Abnormality according to criteria; 4, Not applicable.

JTS survey on 73 Grey Zone External Observations (glossary V 2)



Izumi *et al*, *Congenital Anomalies* 2018;**58**:82-86.

Table 2 List of "Abnormality" based on consideration of Terminology Committee (14 findings)

Region/organ/structure	Observation		Code no.†	Survey result 1/2/3/4‡	Subclassification
General	Subcutaneous hemorrhage		10004	17/0/0/5	Non-structural abnormality
	Skin	Lesion	New	11/1/1/9	Non-structural abnormality
		Tag	New	13/0/1/8	Malformation
Eye	Eye bulge	Large	10028	13/0/3/6	Malformation
		Small	10035	10/0/2/10	Malformation
Paw/digit	Claw	Malpositioned	10082	14/1/3/4	Malformation
		Small	10089	10/0/2/10	Malformation
Tail	Tail	Fleshy tab	10098	15/0/0/7	Malformation
		Long	New	13/1/4/4	Malformation
Trunk	Anus	Large	New	8/1/6/7	Non-structural abnormality
		Small	10118	11/0/4/7	Malformation
	Pelvic region	Narrow	New	12/0/4/6	Malformation
	Trunk	Large	New	10/0/4/8	Malformation
	Umbilicus	Malpositioned	New	15/0/2/5	Malformation

†New: These findings were added in the revised edition (Version 2) (Makris et al. 2009a,b,c), and thus not numbered.
‡Survey results: 1, Abnormality; 2, Variation; 3, Abnormality according to criteria; 4, Not applicable.

?!!

Izumi *et al*, *Congenital Anomalies* 2018;**58**:82-86.

Table 3 List of "Not applicable" findings based on the survey (four findings)

Region/organ/structure	Observation		Code no.†	Survey result 1/2/3/4‡
General	Fetus or pup/neonate	Large	New	4/0/0/18
		Small	New	5/1/1/15
Face	Tongue	Altered surface texture	New	7/0/0/15
	Tooth	Not erupted	New	4/1/1/16

†New: These findings were added in the revised edition (Version 2) (Makris et al. 2009a,b,c), and thus not numbered.
‡Survey results: 1, Abnormality; 2, Variation; 3, Abnormality according to criteria; 4, Not applicable.

Berlin Workshop categories

Malformation:

"*a permanent structural change that is likely to adversely affect the survival or health of the species under investigation*"

Variation:

"*a change that occurs within the normal population under investigation and is unlikely to adversely affect survival or health*"

Undetermined:

"*cannot decide between malformation or variation*"

Not known:

"*not known / not used in the laboratory*"

Chahoud *et al*, *Reproductive Toxicology 1999*;**13**:77-82
Solecki *et al*, *Reproductive Toxicology 2001*;**15**:713-721

OR

Japanese Teratology Society categories

Abnormality:

"*malformation and non-structural abnormality*"

Variation:

"*deviation from normal morphology but considered transient or observed frequently in specific species or strains*"

Depends on severity:

"*either abnormality, variation, or neither of them (normal) depends on severity*"

Not applicable:

"*unable to classify as abnormality or variation in fetuses*"

Izumi *et al*, *Congenital Anomalies* 2018;**58**:82-86.

Berlin Workshops

Malformation ►´*a permanent structural change that is likely to adversely affect the survival or health of the species under investigation*`

Variation ► ´*a change that occurs within the normal population under investigation and is unlikely to adversely affect survival or health*`

Malformation	***Variation***
Permanent	*Transient or Permanent*
*Change **likely** to affect survival or health*	*Change **unlikely** to affect survival or health*
Uncommon within the normal population	*Occurs [commonly ?] within the normal population*

Japanese Teratology Society

Malformation ►´*an uncommon structural change that is deviated from normal morphology of the species or strains induced by developmental disturbance'*

Variation ►´*minor deviations from normal morphology, which was (were) observed in specific species or strains transiently or commonly*`

Malformation	***Variation***
[Permanent and uncommon?]	*Transient [or permanent and common?]*
Uncommon	*Common*
Induced by developmental disturbance...	*Deviation induced by .....?*

Comments on the redefinition of anomaly categories proposed by the JTS

I – Should the proposed binary classification scheme (M or V) be extended to non-structural abnormalities?

Are M and V definitions applicable to non-structural changes or consequences of functional disorders (hemorrhagic, discolored, pale, edema, ...)?

***Non-structural abnormalities do not fit neatly into either "malformation" or "variation" category**.*

*Malformation refers to a **structural defect** in the body due to abnormal embryonic or fetal development, i.e., an irregular, anomalous, abnormal, or faulty structure.*

*Variations are (minor) **deviations from normal morphology**.*

*In principle, **only structural abnormalities** are to be categorized as **Malformations** or **Variations.***

***For regulatory purposes,** the relative importance (health impact on the developing offspring) of non-structural fetal observations should be established on a case-by-case basis by the study authors/rapporteurs.*

Comments on the redefinition of anomaly categories proposed by the JTS

II – Findings the categorization of which into M or V depends on the severity of the change

That lack of information on the severity of the observation precludes an accurate categorization of reported findings (and descriptive terms) is not a new fact. In previous BW surveys, lack of information on severity has been repeatedly identified as a major reason for misclassification and for the existence of a number of descriptive terms in the categorization grey zone.

Observation terms (modifiers) such as "small", "short", "long", "thin", thick", "narrow", "large", dilated" and "bent" cover a wide range of possible appearances, and the abnormal structures described by these terms can range from something that is only marginally outside the "normal range" to something that is severely malformed.

Solecki et al Reprod Toxicol 2001; 15:713-721.

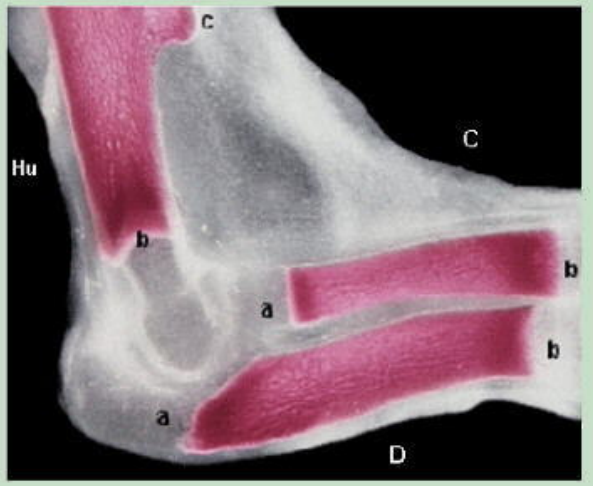
Solecki et al Reprod Toxicol 2003; 17:625-637.

Paumgartten et al Reprod Toxicol 2009; 27:8-13

Solecki et al Reprod Toxicol 2013; 35:48-55.

Solecki et al Reprod Toxicol 2015; 57:140-146.

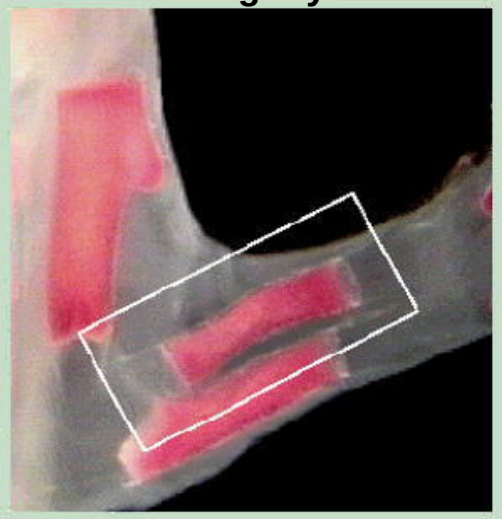
Bent



control

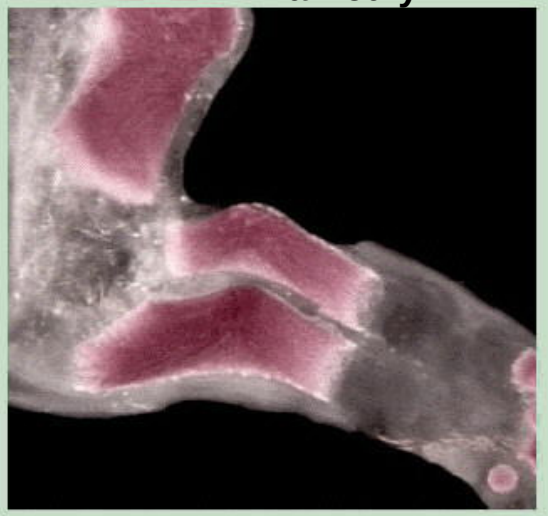
Hu: *os humerus*
c: deltoid process of *os humerus*
C: *os radius*
D: *os ulna*
[Rat fetuses on pregnancy day 21. (Alizarin red S staining)]

slightly



moderately

markedly



Radius bent

In principle, it would be possible to make the description more precise by adding

- ► *more terms (´modifiers`) to describe the changes of the structure (…and….)*
- ► *a grading for severity (modifier of the modifier)*

-Os tibia minimally short with a distal part thin

-Os femur moderately short and minimally bent

-Os Atlas moderately small with a dorsal part markedly thickened

-Os scapula moderately small with a cranial edge moderately misshapened

-and so on

However, this would make descritive terms too long. The use of longer descriptive phrases is not a practical way of reporting findings of DevTox studies.

*Like GZ anomalies, **"Depends on severity"** is not a category of observations. It is just a group of Glossary V2 descriptive terms the categorization of which into **M** or **V** depends on the severity of the actual finding.*

(i .e., more severe ► M, less severe ► V).

*If a majority of survey participants found that categorization of a particular descriptive term of the glossary depends on severity, this indicates that **the actual fetal observation to be described by this term is likely to be considered a M if it is severe or a V if it is mild or less severe**.*

***Categorization** of Glossary descriptive terms **by survey results** thus provides **just a guidance** to investigators, evaluators and regulatory decision makers. We should have in mind that **it is not mandatory** to categorize each and every study finding described by these terms according to the survey results.*

If categorization depends on the severity of the observation and the descriptive term does not provide that information, in the analysis of survey results the term will appear among the Grey zone anomalies. This does not imply that the categorization of an actual study finding into M or V (If it is less severe) by the investigator was uncertain or doubtful.

Comments on the redefinition of anomaly categories proposed by the JTS

III – Are the foreseeable adverse consequences of the fetal observation for postnatal survival and health an important issue to distinguish a M from a V?

In contrast to the BW categorization scheme for fetal observations, the ***new definitions for M and V categories put forward by JTS do not distinguish them based on their probable consequences for survival and health*** *of the species under consideration.*

The elimination of "likely / unlikely having adverse consequences for survival and health" as a key distinction between M and V categories is expected to substantially decrease the number of Grey Zone anomalies because the uncertainty (and lack of knowledge) about the postnatal consequences of the observation is a major obstacle to decide whether findings fall into one or the other category.

Comments on the redefinition of anomaly categories proposed by the JTS

*As far as "**adverse consequences for survival and health**" are concerned, the **uncertainty** hampering categorization into M or V arises from the lack of information on the fate of the **actual fetal observation** described by the glossary term and **not to** (an imperfection of) **the descriptive term itself**.*

*During this series of BW this topic was extensively debated and, from the outset, it became clear that "**grey zone anomalies will never disappear completely**" (Solecki et al, 2001). One of the main reasons for that is the paucity of data on the permanence/transience and health consequences of fetal anomalies (Paumgartten et al, 2009; Solecki et al, 2013).*

In other words, the reduction of Grey Zone anomalies achieved by the JTS survey (external findings) resulted from the redefinition of M and V categories, not from an increased knowledge about the postnatal consequences of fetal observations for survival and health.

Comments on the redefinition of anomaly categories proposed by the JTS

*In my view, "**to (likely/unlikely) adversely affect survival or health" should be kept as a main distinctive feature in the definitions of M and V categories**.*

This makes sense if categorization is intended to be "a valuable tool to indicate the relative importance of the changes observed" and to assist regulatory decision making regarding chemical labelling (Chahoud et al, 1999). It may also be useful for health risk assessment.

The existence of grey zone anomalies when they result from our lack of knowledge on the fate and health consequences of fetal observations is not a great problem.

***Misclassification of findings, and the lack of data on the postnatal consequences of fetal anomalies are indeed the major problems**.*

Concluding remarks

The two-category only scheme as well as the definitions for M and V put forward in 1998 (Chahoud et al 1999) have proved to be a valuable and practical tool for analyzing and translating the information provided by DevTox studies (fetal observations) into regulatory decisions.

There is no need to change the definitions for M and V categories advanced in 1998 (2nd BW).

Recategorization of Grey Zone anomalies *(terms listed in Glossary v1 and v2) identified in previous surveys* ***should result from*** *significant* ***improvements of descriptive terms*** *accuracy* ***and/or from advancement of knowledge regarding their postnatal consequences for survival or health.***

To conduct a new survey for recategorization of Grey Zone anomalies only makes sense if we agree that a substantial progress has been made along these lines.

Concluding remarks

As previously commented, there are two main reasons to call a descriptive term a "grey zone anomaly":

i) categorization into M or V depends on severity; and

ii) information is missing on the postnatal consequences for health.

In the first case, the ***glossary term is a GZ term, not the actual observation****. The study evaluator would categorize it as a M, if it is severe, or as a V if it is less severe. This type of GZ does not seem to be a problem.*

In the second case, categorization of the ***actual observation*** *is indeed a major problem because the* ***consequences for survival or health are not known****. Research is needed to bridge this knowledge gap.*

Concluding remarks

Finally, *we should have in mind that, as far as categorization is concerned, survey reports and so labels such as M, V or "grey zone" are just a guide (not a "jailer") for evaluators/investigators. The categorization of study findings is at their discretion and they should use their own judgement in a case-by-case basis.*

If authors' categorization of a study observation is at variance with that of a majority of (BW) survey participants, the reasons for the divergence could (should) be explained in the study report.

An example along this line is given by the categorization of "zygomatic bone fused with maxilla" as a variation by Chahoud & Paumgartten (2009), although there had been a high agreement (100%) among BW survey participants that it should be a malformation (Solecki et al 2001).

Chahoud & Paumgartten, Environ Res. 2009, 109: 922-929
https://www.devtox.org/nomenclature/ml_organ.php?lan=en

Solecki et al, Reprod. Toxicl. 2001, 15:713-721

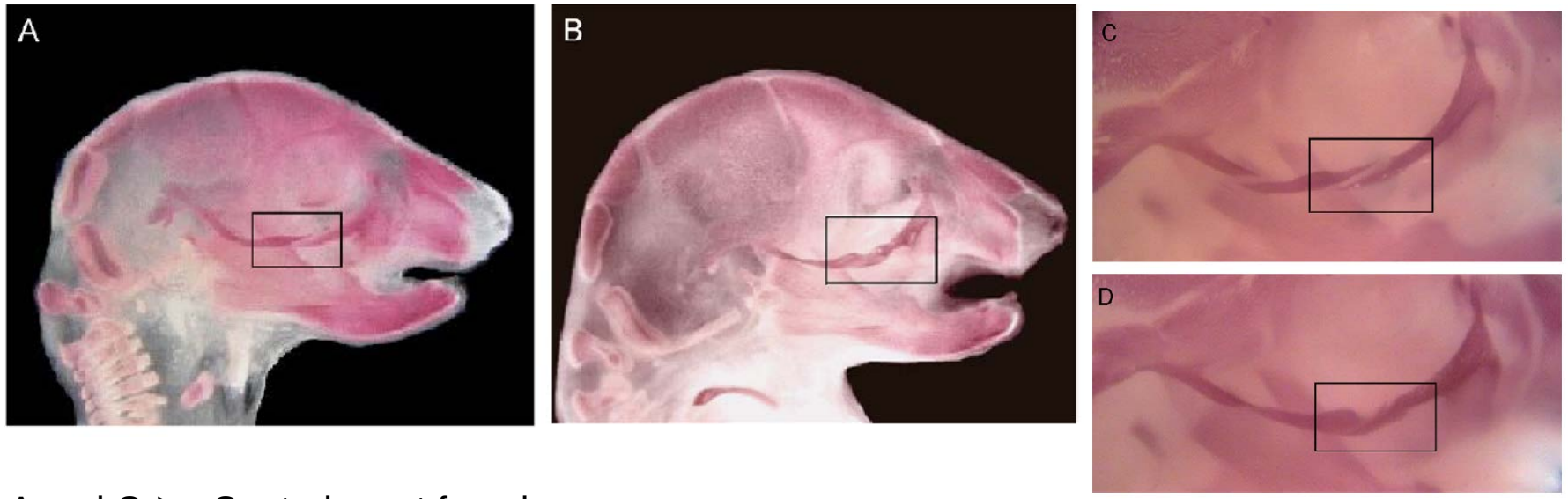
*Does "zygomatic fused" adversely affect the **survival or health** of the species under investigation ?*

This is a key question for classification and health risk assessment. However, it is at times difficult to answer it .

Malformation ►**likely** to adversely affect survival or health

Variation ►**unlikely** to adversely affect survival or health

Rat fetus on **GD 21**. Zygomatic arch Fused – **DevTox classification**: ► **Malformation**



A and C ► Control – not fused

B & D ►Zygomatic bone and zygomatic process (maxilla) fused

Variation because:

- *The incidence of zygomatic bone fused with maxilla in the historical control records for this rat strain (Berlin)♣ is approximately 11%.*
- *Fusion normally occurs later during postnatal growth*
- ***Postnatal consequences of anticipated fusion are unclear***.

Chahoud & Paumgartten, Environ Res. 2009, 109: 922-929
https://www.devtox.org/nomenclature/ml_organ.php?lan=en

Solecki et al, Reprod. Toxicl. 2001, 15:713-721

♣ *In our breeding stock (Wistar rats – FIOCRUZ) zygomatic fused seldom occurs in controls (HC incidence < 0.01%) .*

*“**Owing to this relatively high background occurrence it was assumed that this skeleton structural change does not convey a selective disadvantage for this rat population** and thus, at our laboratory, it has been considered as a variation. This interpretation, however, is at variance with those of most experts who classified zygomatic bone fused, as well as many other fused bones, as malformations (Solecki et al., 2001). It is of note that a fusion of zygomatic bone with maxilla normally occurs later during postnatal growth and **consequences of an anticipated fusion are not clearly understood yet**. At any rate, **zygomatic bone fused was considered in this paper as a variation**.”*

Chahoud and Paumgartten, 2009.

Thank you for your attention