Procedure Severity Assessments in Animal Research: Ethical and Practical Considerations

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1. Introduction to Severity Classification

Recognizing that some animals are sentient beings whose intrinsic value must be respected, *Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes* ("Directive") regulates the procedures that may be carried out on animals and the harm that may be inflicted (Directive, Preamble (12) & (23), Art. 1). The Directive requires prospective, ongoing, and retrospective assessment of procedure severity. Requests to use animals in research must estimate the severity of each procedure to be carried out on each animal during the project (Directive, Art. 15(1)). Actual severity experienced by each animal must be monitored during the project and reported to the authorities after completion (Directive, Art. 39).

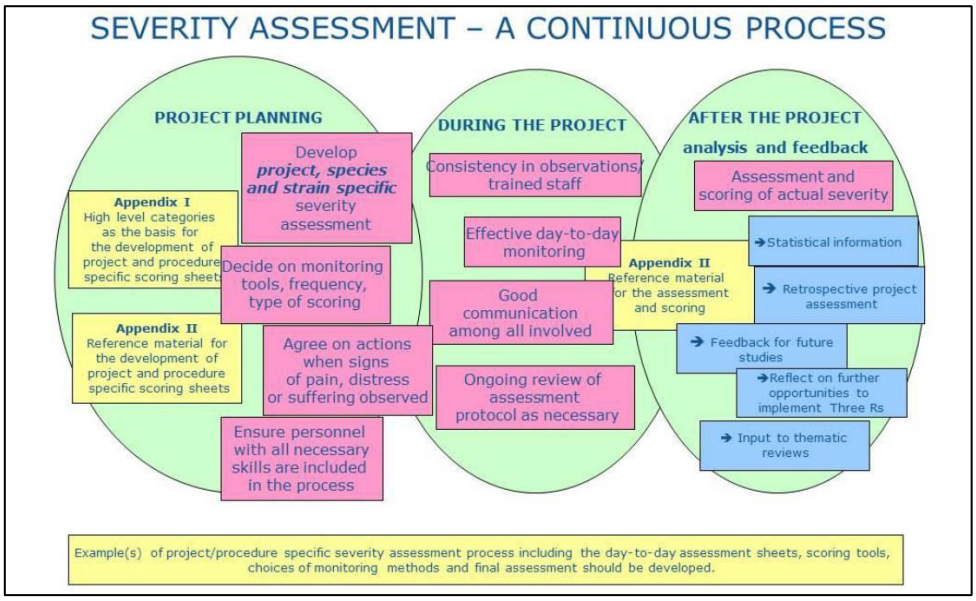

Figure 1: Severity Assessment Process (EU)

Source: EC, Severity Assessment Framework (2018)

Severity assessment is an aspect of implementing the refinement principle, which, together with replacement and reduction, forms part of the "3Rs" of humane experimentation developed by Russell and Burch (1959). The 3Rs, enshrined in the Directive (Art. 4), work together: animals must be replaced by non-sentient alternatives to the greatest extent possible (replacement); insofar as live animals are required, their number must be minimized (reduction); and the "pain, suffering, distress or lasting harm" (Directive, Art. 4(3)) to the remaining animals must be reduced to an "absolute minimum" (refinement) (Russell and Burch, 1992, p. 134). While this note will focus on pain during experimental procedures, severity assessment entails consideration of other factors, such as non-painful negative impacts, *e.g.*, distress caused by behavioural restriction (Directive, Annex VIII).

2. Procedure Severity Assessment ("PSA") Frameworks

The EU PSA framework has four levels: non-recovery, mild, moderate, and severe (Directive, Art. 15). No approval is required for practices less painful than inserting a needle following "good veterinary practice" (Directive, Art. 1(5)(f)). This note (and **Table 1**) will compare the PSA frameworks of Canada, Israel, and Switzerland to that of the EU.

Canada

Canada has an older, five-level PSA framework (CCAC, 1991). Category A procedures (experiments on most invertebrates and live isolates) do not require approval in Canada, but may be reportable in the EU (*e.g.,* as to cephalopods, not conclusively excluded from Category A but protected in the EU) (CCAC, 1991; Directive, Art. 1). Categories B-E increase in severity similarly to the EU PSA's levels (CCAC, 1991). The substantive treatment of non-recovery procedures seems similar, though Canada groups them within Category B ("mild") (CCAC, 1991; Directive, Annex VIII).

The EU is stricter than Canada in some respects. For example, the Directive prohibits stopping the animal from showing pain while withholding analgesia or anesthesia (Directive, Art. 14(3)), while Canada prohibits these practices only in connection with surgical procedures (CCAC, 1989). On the other hand, Canada is more focused on behaviours indicating pain (CCAC, 1991) and expressly mentions the dangers of changing environmental conditions (not addressed in the EU) (CCAC, 1989).

Israel

Israel's PSA framework consists of five severity levels: the lowest (organ collection after euthanasia) is comparable to the EU's "non-recovery" level, and the highest (severe and lasting pain not relieved by analgesics) – to the EU's "severe" level (CAEI, 2017). Notable differences include the reportability in Israel, at Level 2, of procedures would be below the reporting threshold in the EU and the assignment of Level 3 to non-survival major surgery which would fall within the EU's lower "non-recovery" category (Directive, Annex VIII; CAEI, 2017). Israel's PSA shows a greater emphasis on behaviours indicating pain (CAEI, 2017). Finally, the EU requires anesthesia or analgesia whenever the animal is prevented (*e.g.*, by a paralytic) from showing pain (Directive, Art. 14(3)). Israel permits withholding of analgesia if the paralysis is not painful (CAEI, 2017); this can lead to instances of undetected pain.

Switzerland

Switzerland's PSA consists of four levels. The lowest level (interventions that do not cause any pain) includes procedures that would be below the reporting threshold in the EU (FFSVO, 2021(1)). Switzerland does not have a separate non-survival category; its Levels 1-3 roughly correspond to the EU's "mild", "moderate", and "severe" levels (FFSVO, 2021(1)). Both jurisdictions incorporate the duration and intensity of pain and have issued supplementary PSA guidance (EU, Severity; FFSVO, Severity). Like the EU, Switzerland accounts for the aggregate impact of interventions and prohibits induction of paralysis without analgesia and anesthesia (Directive, Art. 14 & Annex VIII; FFSVO, 2020; SAMS, 2005). While Switzerland requires the use of more animals if doing so can significantly reduce individual animal suffering (SAMS, 2005), the Directive does not establish a hierarchy between reduction and refinement (EU Severity, 2013).

3. Classification of Blood Withdrawal From a Giant Pacific Octopus (*Enteroctopus dofleini*) ("GPO")

This note discusses the PSA of a one-time withdrawal from an artery of a GPO of less than 10% of total circulating blood volume without anesthesia ("Withdrawal"). Considering the procedure and the animal together better reflects how severity would be assessed in practice.

In the EU, a one-time withdrawal of less than 10% of circulating volume would qualify as "mild", whereas repeated withdrawals exceeding the 10% threshold where the animal remains conscious and there is no time for blood volume replacement would be considered "moderate" (Directive, Annex VIII). Canada classifies blood withdrawals as Category B ("mild") without further elaboration (CCAC, 1991). In Switzerland, blood withdrawal could be classified as Level 0 (no constraint), Level 1 ("mild"), or Level 2 ("moderate"), depending on factors such as the volumes, intervals, and frequency of the withdrawals, whether anesthesia is needed, the need for and duration of restraint, whether the animal will survive the procedure, and whether the animal is being reused (Severity, Switzerland). In Israel, blood withdrawal classification would range between the EU equivalents of non-reportable and mild depending on the withdrawal site, its volume/amount, and whether anesthesia is needed (CAEI, 2017). The Withdrawal would likely qualify as "mild" in the EU, Canada, and Israel, and "mild" or "moderate" in Switzerland.

However, we cannot rely solely on the nature of the procedure; we must also consider the animal's species and individual characteristics (Directive, Annex VIII; Fenwick *et al.,* 2011). The EU, Switzerland, and (partially) Canada protect cephalopods such as the GPO (CCAC, 1991; Directive, Annex VIII; OPAn, 2008); Israel does not. None of the jurisdictions considered in this note provides species-specific guidance for the GPO. The PSA frameworks' examples of procedures at each severity level and the behavioural signs of pain (typically not even listed for presumably low-severity procedures such as blood withdrawal) seem to be based on the knowledge of pain experience in humans and commonly used vertebrates. For example, the Swiss PSA's example of blood withdrawal qualifying as Level 0 (no constraint) is that of blood collection from a rabbit's ear vein, twice, with a two-week interval, up to 3ml each time, without sedation or restraint (FFSVO, Severity). This classification may be justified as to an apparently simple procedure in a small mammal, but is likely not appropriate for the Withdrawal. Fiorito *et al.* (2015) have proposed guidelines for the use of cephalopods under the Directive, but these are high-level and incomplete (Ponte *et al.*, 2019).

An effective pain PSA requires a thorough understanding of the species, and of the individual animal's background and experience (Andrews *et al.*, 2013). The GPO is sensitive to changes in water temperature, pH, salinity, and chemical composition

(Ponte *et al.*, 2019). Its skin is delicate and easily damaged, and, if removed from the tank, the animal must be irrigated with water to enable it to breathe (Ponte *et al.*, 2019). Its circulatory system, with three hearts (**Fig. 2**), no "readily accessible large superficial blood vessels", and light-blue or colorless blood (making hemorrhage difficult to detect) may complicate the Withdrawal (Fiorito *et al.*, 2015, p. 42).

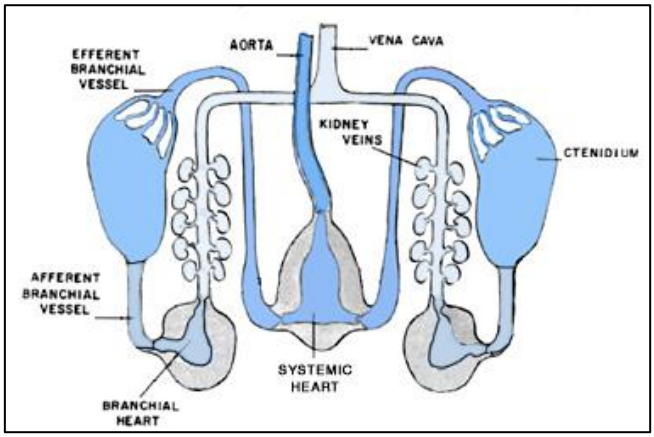

Figure 2: GPO Central Circulatory System (Simplified)

Source: Green, adapted from Johansen and Huston (1962)

It is not clear whether the 10% of the circulating volume threshold (such as that used in the EU and considered appropriate for mammals) would be suitable for the GPO (Fiorito *et al.*, 2015). Fiorito *et al.* (2015) mention studies collecting small amounts of blood from the GPO under anesthesia, which may itself cause pain and whose effect on the GPO is not well-understood (Andrews *et al.*, 2013), but there is no established GPO-appropriate withdrawal volume threshold (Fiorito *et al.*, 2015). Other issues concern the optimal Withdrawal, whether and how the GPO should be restrained, how long the Withdrawal should last, what needle should be used, and what level of pain its insertion could cause (Fiorito *et al.*, 2015; Ponte *et al.*, 2019; Smith *et al.*, 2013). Thus, a procedure that may be short, simple, and relatively painless in a well-understood mammal could become complicated and potentially painful for the GPO. In sum, all four PSAs should be applied to the GPO with great caution, as we can easily miss or misinterpret signs of pain (Andrews *et al.*, 2013; Fiorito *et al.*, 2015; Ponte *et al.*, 2019). The Swiss framework, allowing for the possibility of a moderate classification, seem to be most appropriate for use with the GPO.

4. Ethical Considerations

PSA frameworks aim to continuously improve animal welfare, including setting an upper limit to procedure severity, assessing animal reuse, and determining humane end points (Directive, Art. 15-17 & Annex VIII). Better animal welfare improves science quality, as pain and suffering, particularly unidentified, can confound study results, undermining data usability (Andrews *et al.*, 2013; Smith *et al.*, 2013). PSA is also a key part of ethical review of proposed experiments (Directive, Preamble (12); Fenwick *et al.*, 2011).

Ethical review guides decisions on the moral permissibility of inflicting harm on sentient animals (Fiorito *et al.*, 2015). The aim of ethical review is to authorize only those projects whose expected benefits to "humans, animals or the environment", considering the likelihood that these benefits will be achieved, outweigh the harm to be inflicted on the animals after the implementation of the 3Rs (Directive, Art. 12.2, 27 & 38; Smith *et al.*, 2013). The more significant the harm, the more compelling the justification required(Fenwick *et al.*, 2011).

The ethical theory underlying the harm-benefit analysis and PSA, utilitarianism, seeks to base decisions on balancing the harms and benefits to all affected sentient beings (Palmer and Sandøe, 2011). The morally right approach is that which results in the best outcome overall, and violation of an individual being's interests (*e.g.*, the interest in avoiding pain) is permitted only insofar as it is necessary to achieve this outcome (Palmer and Sandøe, 2011). The utilitarian ethical framework is problematic for at least two reasons. First, humans, who are an interested party, are doing the balancing; this conflict of interest introduces subjectivity and bias (Smith *et al.*, 2013). Humans identify the expected benefits, set up the analytical frameworks, and "define, implement, and monitor" the animal protection measures (SAMS, 2005, p. 1). Bias is exacerbated by the fact that humans have difficulty relating to unfamiliar, less evolutionarily similar species such as the GPO (Mather and Anderson, 2007).

Second, if we do not fully understand a species' experience of pain (Mather and Anderson, 2007), we cannot accurately weigh the impact on their interests. The GPO perception and expression of pain is not well understood (Fiorito *et al.*, 2014). We could consider appearance, behaviour, and physiological indicators, but there is no

grimace scale of the type that exists for many mammals (Dalla Costa *et al.*, 2018), or any other accepted framework (Fiorito *et al.*, 2014). Many gaps also remain in our understanding of GPO analgesia and anesthesia (Fiorito *et al.*, 2014). Thus, we cannot be sure that any PSA framework resolves the ethical conflict between the interest of the GPO and those of human society objectively, accurately, and fairly to the GPO.

Nevertheless, of the PSA frameworks discussed, the author would select the Swiss framework as best meeting our ethical obligations to respect the GPO's sentience regarding pain. The Swiss framework protects the GPO (OPAn, Art. 112) and, in the Withdrawal example, the Swiss PSA framework enabled the most nuanced assessment. A more detailed framework, if accurate, can improve the PSA process by leaving less room for discretion or bias.

In addition, in Switzerland, pain severity is only one aspect of the overall analysis of constraint on the animal; other impacts on the animal's dignity (*e.g.*, humiliation, excessive instrumentalization, and major interference with its appearance or abilities) must also be considered (FFSVO, 2020). Non-pathocentric harms alone can cause a non-painful procedure to be disallowed (FFSVO, 2020). Although the Swiss authorities do not provide cephalopod-related guidance (perhaps because cephalopods are not used in research in Switzerland (FFSVO, 2021(2))), available guidance could be used with the Swiss PSA framework (Fiorito *et al.*, 2015).

The EU framework seems less strict and therefore less GPO-favorable. The Swiss framework provides for a narrow, closed list of permissible animal research purposes: preservation or protection of human or animal life and health; development of new knowledge about fundamental life processes; and environmental protection (FFSVO, 2020). The EU list is much longer and thus less animal-favorable (Directive, Art. 5). Switzerland also seems stricter than the EU with the upper pain threshold. The EU PSA can allow, in exceptional circumstances, procedures that entail severe, unameliorated, and long-lasting pain (Directive, Art. 55). The Swiss PSA does not include a similar provision, and its general approach suggests that these types of procedures would not be allowed. Neither of the remaining PSAs would be a reasonable choice: Israel does not protect cephalopods (IWL, 1994), and the Canadian framework is older, with no cephalopod material available based on this author's research.

5. Conclusion

Accurate assessment of pain is fundamental to meeting our ethical obligations to the experimental animal as a sentient being. The less familiar we are with the species' pain expression, the more questionable our attempts to balance its presumed pain level with the interests asserted in support of the experiment. Assuming that a particular type of procedure would generate specific pain level in one species based on its known effect on another is not sufficient. A good understanding of how that species, and that particular animal, experiences and expresses pain is required (Fiorito *et al.*, 2015). Absent such an understanding, the animal should be given every benefit of the doubt, particularly as it is humans who carry out the harm-benefit analysis.

PSA frameworks, while well-intentioned, can raise practical concerns. Insufficiently specific PSA guidance can lead to inappropriate animal reuse and inconsistent PSA assessments across jurisdictions (Smith *et al.*, 2018). The fact that only a few jurisdictions have PSA frameworks and PSA grading differs by jurisdictions can undermine public trust and therefore acceptance of animal experimentation. Using animal data from a jurisdiction with no or deficient PSA process raises ethical concerns (lack of moral justification for pain infliction) and scientific validity issues (data generated by animals potentially experiencing pain or distress are unreliable). Scientific validity issues can also arise with respect to higher severity procedures in which analgesia or anesthesia are insufficient or not given: the greater the animal's pain, the more questionable the data generated (Smith *et al.*, 2018).

Table 1: Comparison of the PSA Frameworks of the EU, Canada, Israel, and Switzerland[1]

EU **(Four severity levels)** (Directive, Art. 15(1) and Annex VIII)	**Canada** **(Invasiveness categories, A-E)** (CCAC, 1991)	**Israel** **(Severity levels, 1-5)** (CAEI, 2021; CAEI, 2017; Kalman *et al.*, 2018, p. 217)	**Switzerland** **(Constraint levels, 0-3)** (OPAn, 2008, Art. 24[2]; SSVO, 2021; SSVO, 2020; SSVO, Severity)
Below reporting threshold: procedures which may not "*cause the animal a level of pain, suffering, distress or lasting harm equivalent to or higher than, that caused by the introduction of a needle in accordance with good veterinary practice*" (Art. 3(1)). Conducting several below-threshold procedures may cause the reportability threshold to be crossed (Ponte *et al.*, 2019; Annex VIII).	**Category A** (below reporting threshold): experiments on most invertebrates (excl. cephalopods) and live isolates. **Category B** (reportable): experiments that cause little or no stress or discomfort. Examples (no stress/discomfort): "*short periods of food and/or water deprivation equivalent to periods of abstinence in nature*".	**Level 2** (reportable): Experiments causing slight temporary discomfort or stress (or slight pain that the animal can avoid). Israel's Council for Animal Experiments clarifies that this severity level entails procedures that cause harm that does not exceed that resulting from the introduction of a needle into a healthy animal. While Israeli guidance considers procedures at Levels 1 and 2 to be non-reportable in the EU, the analogy is imperfect. For example, procedures causing harm equal to the introduction of a syringe would be reportable in the EU, as would be organ collection.	**Constraint level 0/No constraint** (reportable): The experiment does not expose the animal to pain, suffering, or injury, does not cause fear, and does not undermine the animal's health. Example: observational study;[3] "*[s]ampling of blood … without sedation, at intervals and frequencies or in volumes imposing no constraint on the animals (no prolonged restraining measures, no other interventions or previous administrations of test substances).* "*Collection of body fluids … under deep general anaesthesia directly followed by*

[1] The cells corresponding to the possible classification of the Withdrawal under various PSA framework are highlighted in yellow.
Due to the differences between the assessed jurisdictions' PSA frameworks, it is not possible to fully align the severity categories. This table seeks to align the PSA frameworks of the other jurisdictions to that of the EU.

[2] As English is not an official language in Switzerland, this author used the following English language summary (cited in full in the references list) provided by the Swiss authorities: https://www.blv.admin.ch/blv/en/home/tiere/tierversuche/schweregrad-gueterabwaegung.html.

[3] Here and throughout, for Switzerland: detailed examples for each severity level are available in the document referred to as "FFSVO, Severity", included in the bibliography. Only the examples relevant to blood withdrawal are reproduced.

EU **(Four severity levels)** (Directive, Art. 15(1) and Annex VIII)	**Canada** **(Invasiveness categories, A-E)** (CCAC, 1991)	**Israel** **(Severity levels, 1-5)** (CAEI, 2021; CAEI, 2017; Kalman *et al.*, 2018, p. 217)	**Switzerland** **(Constraint levels, 0-3)** (OPAn, 2008, Art. 24[2]; SSVO, 2021; SSVO, 2020; SSVO, Severity)
			euthanasia in animals not previously subjected to any intervention. *Examples: Collection of blood samples from the ear vein of the rabbit, twice with an interval of 14 days, 3 ml on each occasion*" (FFSVO, Severity, p. 15).
Non-recovery: entire procedure is performed under general anesthesia from which the animal does not recover.	**Category B**: experiments that cause little or no stress or discomfort. Examples: "*acute non-survival studies in which the animals are completely anesthetized and do not regain consciousness; approved methods of euthanasia following rapid unconsciousness, such as anesthetic overdose, or decapitation preceded by sedation or light anesthesia*". In this author's opinion, Canada's Category B includes both procedures that would be classified as "non-recovery" and those that would be classified as "mild" in the EU. An attempt has been made to split accordingly the examples provided by the Canadian authorities for this category.	**Level 1**: Collection of organs from animals not used for any experimental procedures and euthanized using established practices.	While no separate category exists, some examples from the FFSVO, Severity document list, at constraint level 0, non-survival procedures (*e.g.*, organ or body part collection under general anesthesia) which would appear to be equivalent to the EU's "non-recovery" category.

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Mild: procedures likely to cause "*short-term mild pain, suffering or distress*" and those that do not significantly impair the animal's welfare. Examples: *"(a) administration of anaesthesia except for the sole purpose of killing;* *(b) pharmacokinetic study where a single dose is administered and a limited number of blood samples are taken (totalling < 10 % of circulating volume) and the substance is not expected to cause any detectable adverse effect;* *(c) non-invasive imaging of animals (e.g. MRI) with appropriate sedation or anaesthesia;* *(d) superficial procedures, e.g. ear and tail biopsies, non-surgical subcutaneous implantation of mini-pumps and transponders;* *(e) application of external telemetry devices that cause only minor impairment to the*	**Category B**: experiments that cause little or no stress or discomfort. Examples: maintaining animals in actual/simulated commercial production management systems, "*the short-term and skillful restraint of animals for purposes of observation or physical examination; blood sampling; injection of material in amounts that will not cause adverse reactions by the following routes: intravenous, subcutaneous, intra- muscular, intraperitoneal, or oral, but not intrathoracic or intracardiac (Category C);... short periods of food and/or water deprivation equivalent to periods of abstinence in nature*". **Category C**: experiments that cause minor short-term pain or stress. Examples: "*cannulation or catheterization of blood vessels or body cavities under anesthesia; minor surgical procedures under anesthesia, such as biopsies, laparoscopy; short periods of restraint beyond that for simple observation or examination, but consistent with minimal*	Certain **Level 2** procedures, in this author's opinion (which diverges from the Israeli authority's view that all Level 2 procedures would be non-reportable in the EU). Examples: tail tip sampling, blood withdrawals from peripheral vessels (up to 10% of circulating blood or 1% of the animal's weight); these would be reportable in the EU. **Level 3**: Experiments causing slight stress or short-term pain. Examples: "*nonsurvival major surgery; cannulation; minor survival surgery; blood withdrawal under anesthesia from the retroorbital sinus or from the heart; restraint for short periods; water or food restriction for less than 12h a day*" (Kalman *et al.*, 2018, p. 217) Non-survival major surgery, falling under Level 3 in Israel, would be classified as "non-recovery" in the EU.	**Constraint Level 1/Slight**: intervention or handling causes slight pain or injury or slightly undermines the animal's health. Examples: "*Lege artis collection of blood ... with or without sedation, at intervals and frequencies imposing mild short-term constraint on the animals with non-toxic doses of test substances, slightly prolonged reduced housing conditions.* *Examples: Several blood samples from the tail vein, saphenous vein or sublingual vein in the mouse and rat within 24 hours*" (FFSVO, Severity, p. 15).

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animals or minor interference with normal activity and behaviour; *(f) administration of substances by subcutaneous, intramuscular, intraperitoneal routes, gavage and intravenously via superficial blood vessels, where the substance has no more than mild impact on the animal, and the volumes are within appropriate limits for the size and species of the animal;* *(g) induction of tumours, or spontaneous tumours, that cause no detectable clinical adverse effects (e.g. small, subcutaneous, non-invasive nodules);* *(h) breeding of genetically altered animals, which is expected to result in a phenotype with mild effects;* *(i) feeding of modified diets, that do not meet all of the animals' nutritional needs and are expected to cause mild clinical abnormality within the time-scale of the study;*	*distress; short periods of food and/or water deprivation which exceed periods of abstinence in nature; behavioral experiments on conscious animals that involve short-term, stressful restraint; exposure to non-lethal levels of drugs or chemicals. Such procedures should not cause significant changes in the animal's appearance, in physiological parameters such as respiratory or cardiac rate, or fecal or urinary output, or in social responses.* *During or after Category C studies, animals must not show self-mutilation, anorexia, dehydration, hyperactivity, increased recumbency or dormancy, increased vocalization, aggressive defensive behavior or demonstrate social withdrawal and self-isolation*".		

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(j) short-term (< 24h) restraint in metabolic cages; *(k) studies involving short-term deprivation of social partners, short-term solitary caging of adult rats or mice of sociable strains;* *(l) models which expose animals to noxious stimuli which are briefly associated with mild pain, suffering or distress, and which the animals can successfully avoid; (m) a combination or accumulation of the following examples may result in classification as 'mild': (i) assessing body composition by non-invasive measures and with minimal restraint; (ii) monitoring ECG with non-invasive techniques with minimal or no restraint of habituated animals; (iii) application of external telemetry devices that are expected to cause no impairment to socially adapted animals and do not interfere with normal activity and behaviour; (iv) breeding genetically altered animals which are expected to have no clinically detectable adverse phenotype; (v) adding inert markers in the diet to follow passage of digesta; (vi) withdrawal*			

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of food for < 24h in adult rats; (vii) open field testing".			
Moderate: procedures likely to cause *"short-term moderate pain, suffering, or distress, or long-lasting mild pain, suffering, or distress"* and those that are likely to moderately impair the animal's welfare. *Examples:* *"a) frequent application of test substances which produce moderate clinical effects, and withdrawal of blood samples (> 10 % of circulating volume) in a conscious animal within a few days without volume replacement;* *(b) acute dose-range finding studies, chronic toxicity/carcinogenicity tests, with non-lethal end-points;* *(c) surgery under general anaesthesia and appropriate analgesia, associated with post surgical pain, suffering or impairment of general condition. Examples include: thoracotomy, craniotomy, laparotomy, orchidectomy, lymphadenectomy,*	**Category D**: experiments that cause "*moderate to severe distress or discomfort*". Examples: "*major surgical procedures conducted under general anesthesia, with subsequent recovery; prolonged (several hours or more) periods of physical restraint; induction of behavioral stressors such as maternal deprivation, aggression, predator-prey interactions".* In this author's opinion, Canada's Category D includes both procedures that would be classified as "moderate" and those that would be classified as "severe" in the EU. An attempt has been made to split accordingly the examples provided by the Canadian authorities for this category.	**Level 4**: Experiments causing medium pain or distress, which is alleviated by analgesics. Examples: "*major survival surgeries where animals receive analgesics; local nonmetastatic tumors where animals receive analgesics; restraining animals for over 60 min; restriction of water or food for over 12h during the animal's activity phase; significant changes in environmental parameters (temperature, lighting); procedures that cause sensory or motor damage or severe and constant anatomical and/or physiological changes; use of complete Freund's adjuvant*" (Kalman *et al.*, 2018, p. 217).	**Constraint Level 2/Moderate**: intervention or handling causes short-term moderate or medium-to-long term slight pain, suffering or injury; short-term moderate fear; or short-to-medium term severe health impairment. Examples: "*Sampling of blood in volumes and at intervals and frequencies causing moderate short-term constraint on the animals[.] Sampling of body fluids (in relatively large quantities, in relatively large numbers or at relatively short intervals) after administration of pharmacologically active substances (no toxic doses, no other interventions, no prolonged restraining measures).* *Examples: Repeated daily collection of blood samples from the tail vein in rats over five days to determine the course of hormone levels*" (FFSVO, Severity, p. 16).

EU **(Four severity levels)** (Directive, Art. 15(1) and Annex VIII)	**Canada** **(Invasiveness categories, A-E)** (CCAC, 1991)	**Israel** **(Severity levels, 1-5)** (CAEI, 2021; CAEI, 2017; Kalman *et al*., 2018, p. 217)	**Switzerland** **(Constraint levels, 0-3)** (OPAn, 2008, Art. 24[2]; SSVO, 2021; SSVO, 2020; SSVO, Severity)
thyroidectomy, orthopaedic surgery with effective stabilisation and wound management, organ transplantation with effective management of rejection, surgical implantation of catheters, or biomedical devices (e.g. telemetry transmitters, minipumps etc.); *(d) models of induction of tumours, or spontaneous tumours, that are expected to cause moderate pain or distress or moderate interference with normal behaviour;* *(e) irradiation or chemotherapy with a sublethal dose, or with an otherwise lethal dose but with reconstitution of the immune system. Adverse effects would be expected to be mild or moderate and would be short-lived (< 5 days);* *(f) breeding of genetically altered animals which are expected to result in a phenotype with moderate effects;* *(g) creation of genetically altered animals through surgical procedures;*			

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(h) use of metabolic cages involving moderate restriction of movement over a prolonged period (up to 5 days); *(i) studies with modified diets that do not meet all of the animals' nutritional needs and are expected to cause moderate clinical abnormality within the time-scale of the study;* *(j) withdrawal of food for 48 hours in adult rats;* *(k) evoking escape and avoidance reactions where the animal is unable to escape or avoid the stimulus, and are expected to result in moderate distress".*			
Severe: procedures likely to cause "*severe pain, suffering, or distress or long-lasting moderate pain, suffering or distress*" and those that are likely to severely impair the animal's welfare. Examples: *"a) toxicity testing where death is the end-point, or fatalities are to be expected and severe pathophysiological states are*	**Category D**: experiments that cause "*moderate to severe distress or discomfort*". Examples: "*procedures which cause severe, persistent or irreversible disruption of sensorimotor organization; the use of Freund's Complete Adjuvant[;] induction of anatomical and physiological abnormalities that will result in pain or distress; the exposure of an animal to noxious stimuli from which escape is*	**Level 5**: Experiments causing severe and lasting pain or distress not alleviated by analgesics. Examples: "*metastatic tumors or experiments in which the endpoint is death*" (Kalman *et al*., 2018, p. 217). Requires justification as to non-use of analgesics.	**Constraint Level 3/Severe**: intervention or handling causes "*short-term moderate or medium- to long-term slight pain, suffering or injury, short-term moderate fear or short to medium-term severe impairment*" of the animal's health. Examples: transplanting aggressive tumours.

EU **(Four severity levels)** (Directive, Art. 15(1) and Annex VIII)	**Canada** **(Invasiveness categories, A-E)** (CCAC, 1991)	**Israel** **(Severity levels, 1-5)** (CAEI, 2021; CAEI, 2017; Kalman *et al.*, 2018, p. 217)	**Switzerland** **(Constraint levels, 0-3)** (OPAn, 2008, Art. 24[2]; SSVO, 2021; SSVO, 2020; SSVO, Severity)
induced. For example, single dose acute toxicity testing (see OECD testing guidelines); *(b) testing of device where failure may cause severe pain, distress or death of the animal (e.g. cardiac assist devices);* *(c) vaccine potency testing characterised by persistent impairment of the animal's condition, progressive disease leading to death, associated with long-lasting moderate pain, distress or suffering;* *(d) irradiation or chemotherapy with a lethal dose without reconstitution of the immune system, or reconstitution with production of graft versus host disease;* *(e) models with induction of tumours, or with spontaneous tumours, that are expected to cause progressive lethal disease associated with long-lasting moderate pain, distress or suffering. For example tumours causing cachexia, invasive bone tumours, tumours resulting in metastatic*	*impossible; the production of radiation sickness; exposure to drugs or chemicals at levels that impair physiological systems*". Procedures in Category D "*should not cause prolonged or severe clinical distress as may be exhibited by a wide range of clinical signs, such as marked abnormalities in behavioral patterns or attitudes, the absence of grooming, dehydration, abnormal vocalization, prolonged anorexia, circulatory collapse, extreme lethargy or disinclination to move, and clinical signs of severe or advanced local or systemic infection, etc.*" **Category E**: experiments that cause unanesthetized conscious animals severe pain near/at/above the tolerance threshold. Examples: surgical procedures, "*exposure to noxious stimuli or agents whose effects are unknown; exposure to drugs or chemicals at levels that (may) markedly impair physiological systems and which cause death, severe pain, or extreme distress; completely new biomedical experiments which have a high degree of invasiveness,*		The Swiss limits for this severity level seem to be lower than those in Canada; it seems likely that Switzerland would more readily prohibit certain experiments than Canada, no matter the societal goals (SAMS, 2005).

EU **(Four severity levels)** (Directive, Art. 15(1) and Annex VIII)	**Canada** **(Invasiveness categories, A-E)** (CCAC, 1991)	**Israel** **(Severity levels, 1-5)** (CAEI, 2021; CAEI, 2017; Kalman *et al*., 2018, p. 217)	**Switzerland** **(Constraint levels, 0-3)** (OPAn, 2008, Art. 24[2]; SSVO, 2021; SSVO, 2020; SSVO, Severity)
spread, and tumours that are allowed to ulcerate; *(f) surgical and other interventions in animals under general anaesthesia which are expected to result in severe or persistent moderate postoperative pain, suffering or distress or severe and persistent impairment of the general condition of the animals. Production of unstable fractures, thoracotomy without adequate analgesia, or trauma to produce multiple organ failure;* *(g) organ transplantation where organ rejection is likely to lead to severe distress or impairment of the general condition of the animals (e.g. xenotransplantation);* *(h) breeding animals with genetic disorders that are expected to experience severe and persistent impairment of general condition, for example Huntington's disease, Muscular dystrophy, chronic relapsing neuritis models;*	*behavioral studies about which the effects of the degree of distress are not known; use of muscle relaxants or paralytic drugs without anesthetics; burn or trauma infliction on unanesthetized animals; a euthanasia method not approved by the CCAC; any procedures (e.g., the injection of noxious agents or the induction of severe stress or shock) that will result in pain which approaches the pain tolerance threshold and cannot be relieved by analgesia (e.g., when toxicity testing and experimentally-induced infections disease studies have death as the endpoint*". The Canadian Ethics Guidelines advise that death should not be the endpoint; alternative endpoints should be set based on the signs of pain or distress.		

EU **(Four severity levels)** (Directive, Art. 15(1) and Annex VIII)	**Canada** **(Invasiveness categories, A-E)** (CCAC, 1991)	**Israel** **(Severity levels, 1-5)** (CAEI, 2021; CAEI, 2017; Kalman *et al*., 2018, p. 217)	**Switzerland** **(Constraint levels, 0-3)** (OPAn, 2008, Art. 24[2]; SSVO, 2021; SSVO, 2020; SSVO, Severity)
(i) use of metabolic cages involving severe restriction of movement over a prolonged period; *(j) inescapable electric shock (e.g. to produce learned helplessness);* *(k) complete isolation for prolonged periods of social species e.g. dogs and non-human primates;* *(l) immobilisation stress to induce gastric ulcers or cardiac failure in rats;* *(m) forced swim or exercise tests with exhaustion as the end-point".*			

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