

## Guidelines for prospective assessment of severity scores of different procedures and disease models

In case during the experiments, unexpected complications occur, the severity score should be retrospectively adapted depending on the actual pain/stress the animals experienced. This can be estimated based on the notes in the animal's welfare diary

TYPE	MODEL	SUBCATEGORY	SEVERITY SCORE	COMMENTS
Tumor models	Subcutaneous and mammary fat pad tumor models (<2cm <sup>3</sup> )	Single tumor without ulceration causing no clinical symptoms	Mild	- Maximal tumor volume has been adapted to 2000 mm <sup>3</sup> instead of 1500 mm <sup>3</sup> previously
		Multiple tumors without ulceration causing no clinical symptoms	Mild	- in case of multiple tumors, max combined tumor volume should be <2cm <sup>3</sup>
		Ulcerated tumor with < 10% of tumor volume ulcerated	Moderate	
		Ulcerated tumor with > 10% of tumor volume ulcerated	Severe	
	Intraperitoneal tumor model	Tumor model at early stage causing no clinical symptoms	Mild	
		Tumor model not necessitating ascites formation and animals are killed at first appearance of ascites	Moderate	- Presence of ascites is considered as a humane endpoint.
		Tumor model requiring ascites formation and/or ascitic drainage	Severe	- Max 5 ascitic drainages are allowed.

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Tumor models	Orthotopic tumor models requiring surgical implantation (e.g. brain, liver, pancreas, colon tumors)		Moderate	-Animals are killed at first appearance of clinical symptoms caused by the tumor model (e.g. headtilt, seizures, circling, diarrhea, bloody stool, constipation, tachypnea, limping, paresis, paralysis, icterus..) and wound. heals normally.
	Orthotopic oral tumor models (e.g. tongue tumors)	Tumor model causing no clinical symptoms	Mild	
		Tumor model causing mild impairment for eating/drinking	Moderate	- Animals are killed at first appearance of severe impairment for eating/drinking .
	Orthotopic lung tumor model (via minimally-invasive inoculation)	Tumor model causing no clinical symptoms	Mild	
		Tumor model causing mild respiratory symptoms	Moderate	- Animals are killed at first appearance of severe respiratory symptoms (e.g. dyspnea, cyanosis).
		Tumor model causing severe respiratory symptoms	Severe	
	Metastatic models (e.g. i.v. injected tumor models)	Widespread metastatic tumor model causing no clinical symptoms	Mild	
		Widespread metastatic tumor model causing mild clinical symptoms	Moderate	- Animals are killed at first appearance of severe clinical symptoms.
		Widespread metastatic tumor model causing severe clinical symptoms (e.g. cachexia)	Severe	

TYPE	MODEL	SUBCATEGORY	SEVERITY SCORE	COMMENTS
Tumor models	Metastatic models (e.g. i.v. injected tumor models)	Tumor models requiring surgical removal of s.c. primary tumor for development of local metastasis, causing no or mild clinical symptoms	Moderate	- Animals are killed at first appearance of severe clinical symptoms.
			Severe	
	Multiple myeloma (MM) tumor model	Animals killed without clinical symptoms (paresis, paralysis)	Mild	
		Animals killed at first appearance of clinical symptoms (paresis, paralysis)	Moderate	- Animals are killed at first appearance of severe clinical symptoms such as paresis or paralysis.
	Animals with major clinical symptoms (paresis, paralysis)	Severe		
Surgical procedures	Minor surgical interventions (e.g. subcutaneous implantations or intranodal injections)		Mild	- Interventions requiring only minor skin incision
	General surgical procedures		Moderate	- Not applicable for bone surgery, transplantations etc --> severe
	Dorsal skinfold window placement		Moderate	
Behavioral tests	Tests necessitating brief handling of the animals , minor changes in housing conditions or food/water availability.	Grip strength, rotarod, elevated plus maze, T/Y/Barnes maze, social interaction, novel object recognition, open field exploration, Marble burying test, etc.	Mild	"- Food restriction for 12h (overnight), causing up to 20% BW loss; - Multiple mild procedures remain mild, but phenotype of the mice might influence total severity (e.g. anxious mouse model)"

TYPE	MODEL	SUBCATEGORY	SEVERITY SCORE	COMMENTS
Behavioral tests	Tests measuring despair, repeated non-avoidable mild electric shocks of short duration, moderate changes in housing conditions or food/water availability	Contextual fear conditioning (e.g. cage stressors for 4 weeks), cue fear conditioning, treadmill with mild electrical shocks, forced swim test without exhaustion etc.	Moderate	- Multiple moderate procedures remain moderate, but phenotype of the mice might influence total severity (e.g. anxious mouse model)
	Tests with exhaustion as endpoint	Forced swim test with exhaustion as endpoint	Severe	
	Animal restraintment as stressor	Repetitive animal restraintment-model in falcon tube to induce stress	Moderate	
Pain experiments	Experiments causing mild, short-term pain with no long-lasting effects	Hot plate test, tail flick test, tail immersion test, performed maximally 5 times over a short period of time (48h).	Mild	- Max. slight tissue swelling - Mice are allowed to react to escape pain - Max 5 such tests performed over short period of time (48h interval)
	Experiments causing mild, short-term pain with no long-lasting effects	Multiple (>5 tests) mild pain experiments performed over a short period of time	Moderate	
	Nerve-crush/ligation of sciatic nerve		Moderate	
	Footpad injection with CFA		Severe	
Non-invasive tissue collection	Non-invasive tissue collections causing only brief handling of animals	Faeces collection, urine collection, swaps, etc.	No harm	

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Blood collection methods	Blood collection methods	Retro-orbital bleedings without complications	Mild	- Retro-orbital bleedings can only be approved with specific motivations
		Retro-orbital bleedings with minor clinical complications (for training)	Moderate	- Retro-orbital bleedings are limited to 1x per eye, and min; 2 weeks interval
		Blood collection methods respecting basic volumes (single and multiple blood collections over a long time interval)	Mild	
		Multiple (>5) blood collections respecting basic volumes over a short period of time (<48h)	Moderate	- Depending on the blood collection method, the max blood volume of 10% of TBV is easily reached after 2-3 procedures. - if animals are under continuous anesthesia, the blood collections are only counted once per anesthesia session
		Single and multiple blood collection methods for large volumes, with fluid replacement	Moderate	
		Multiple blood collections via tail pricking (few µL) or removal of tail end crust	Mild	
Oral gavage	Oral gavage	Single and repetitive oral gavages with min. 24h interval	Mild	
		Repetitive oral gavages with less than 24h interval	Moderate	

TYPE	MODEL	SUBCATEGORY	SEVERITY SCORE	COMMENTS
Systemic injections methods	Systemic injections (i.v., s.c., i.p. etc)	Single and repetitive injections over a long time interval	Mild	
		Multiple injections (>5) over a short period of time (<48h)	Moderate	- if animals are under continuous anesthesia, the injections are only counted once per anesthesia session
Functional tests	Functional tests	Insulin tolerance test (single or repetitive)	Mild	- 4-6h fasting, ip injection, blood collection via tail end crust
		Glucose tolerance test (single or repetitive)	Moderate	- overnight fasting, ip injection, blood collection via tail end crust
Parasite models	African trypanosomias	Aggressive model in which mice are killed before the onset of clinical symptoms	Mild	- Model typically lasts about 4 weeks, with no clinical symptoms in week 1-3; In week 4 severe anemia occurs
		Aggressive model in which mice are killed at first appearance of clinical symptoms	Moderate	- Effects on locomotor activity, > 20%BW loss
		Aggressive model in which mice are successfully treated before clinical symptoms become severe	Moderate	- If mice are not successfully treated, the treatment was not effective. As no intervention (killing) took place to relieve the pain in these mice was applied, their pain score should be scored severe.
		Chronic model in which mice are killed before the onset of clinical symptoms	Mild	- Model typically lasts about 3-4 months, with no clinical symptoms in month 1-2; In month 3-4 severe anemia occurs as well as severe hepatosplenomegaly (significant BW gain)
		Chronic model in which mice are killed at first appearance of clinical symptoms	Moderate	- Clinical symptom includes steep BW increase

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Parasite models	African trypanosomiasis	Chronic model in which mice are successfully treated before clinical symptoms become severe	Moderate	- If mice are not successfully treated, the treatment was not effective. As no intervention (killing) took place to relieve the pain in these mice was applied, their pain score should be scored severe.
Abnormal housing conditions	Solitary housing	Solitary housing without sensory deprivation (still olfactory, auditory OR visual contact), independently of the period	Mild	- Also for housing in IVC cages
		Solitary housing with sensory deprivation (no olfactory, auditory or visual contact) <24h	Mild	- Also for housing in IVC cages
		Solitary housing with sensory deprivation (no olfactory, auditory or visual contact) 1-5 days	Moderate	
		Solitary housing with sensory deprivation (no olfactory, auditory or visual contact) >5 days	Severe	
	Solitary housing - metabolic cage	Solitary housing in metabolic cages <24h	Mild	
		Solitary housing in metabolic cages 1-5 days	Moderate	
		Solitary housing in metabolic cages >5 days	Severe	