

	Extraction/Sample Preparation		Methodology		TAT		Sample Requirements	
	JMHLW	ISO*	JMHLW	ISO	JMHLW	ISO	JMHLW	ISO*
<b>Cytotoxicity</b>	24 hours in EMEM + 10% FBS	24 hours in EMEM + 5% FBS	Dilute suspension of cells is concurrently plated with the extract and incubated for 7 days.	Extract is added to cell monolayer and observed up to 72 hrs.	35 days	21 days	2g or 120cm <sup>2</sup>	4g, 120 cm <sup>2</sup> or 60 cm <sup>2</sup>
<b>Sensitization</b>	Exaggerated extract in methanol/acetone with terminal evaporation	72 hrs. / 37°C extract in saline and cottonseed oil	11 test animals, 6 negative and 6 positive control. Concurrent positive control group required. Terminal weights.	11 test, 6 negative control. Positive control validation run every six months.	84 Days	56 Days	10 grams	4g, 120 cm <sup>2</sup> or 60 cm <sup>2</sup> (x6)
<b>Genotoxicology Bacterial Reverse Mutation Test</b>	Exaggerated extract in methanol/acetone with terminal evaporation	72 hrs. / 37°C extract in saline and DMSO	Extracts are combined with agar and bacteria. Plates are incubated for 72 hours and colonies enumerated.		45 days	31 days	4g, 120 cm <sup>2</sup> or 60 cm <sup>2</sup> (x2)	4g, 120 cm <sup>2</sup> or 60 cm <sup>2</sup> (x2)
<b>Genotoxicology – Chromosome Aberration</b>	Exaggerated extract in methanol/acetone with terminal evaporation	72 hrs. / 37°C extract in saline and DMSO	Extracts are applied to a cell monolayer for up to 20 hours. Cells are harvest and prepared for microscopic examination of gross chromosomal damage.		67 days	53 days	4g, 120 cm <sup>2</sup> or 60 cm <sup>2</sup> (x2)	4g, 120 cm <sup>2</sup> or 60 cm <sup>2</sup> (x2)
<b>Hemolysis</b>	Direct exposure for 1 hr., 2 hrs and 4 hrs.	Direct exposure for 3 hrs.	Test article is placed into dilute solution of rabbit blood for 1, 2 and 4 hrs. Detection reagent is added to the solution. After centrifugation, optical density is scanned.	Test article is placed into dilute solution of rabbit blood for 3 hrs. Detection reagent is added to the solution. After centrifugation, optical density is scanned.	35 days	21 days	4g, 120 cm <sup>2</sup> or 60 cm <sup>2</sup> (x3)	4g, 120 cm <sup>2</sup> or 60 cm <sup>2</sup> (x2)
<b>Stimulation – Intracutaneous Irritation</b>	Extraction methods identical		2 rabbits dosed intracutaneously with each extract. Observations and scoring up to 72 hours. JMHLW requires photos of sites at dosing and all scoring periods and terminal weight.		31 days	31 days	4g, 120 cm <sup>2</sup> or 60 cm <sup>2</sup> (x2)	4g, 120 cm <sup>2</sup> or 60 cm <sup>2</sup> (x2)
<b>Stimulation – Primary Skin</b>	Standard extraction	One-inch-square pieces of test article	6 rabbits per extract, at least 2 extracts, patched for 24 hours, scratched and unscratched skin, terminal weight and photos of sites.	3 rabbits, patches are applied for 4 or 24 hrs., removed and scored.	32 days	32 days	4g, 120 cm <sup>2</sup> or 60 cm <sup>2</sup> (x2)	2 cm <sup>2</sup> x 2 cm <sup>2</sup> (x7)
<b>Implant</b>	Preparation methods identical		4 rabbits, males only. Max. of 4 test articles per rabbit.	3 rabbits, either sex. 4-6 test articles can be implanted.	Same implant duration		NA	2 cm <sup>2</sup> x 2 cm <sup>2</sup> (x7)
<b>Acute Systemic Toxicity</b>	121°C extractions must be performed in an autoclave.	ISO does not specifically state how 121°C extractions should be done.	Necropsy required at study termination. Body weight loss indicative of toxicity if statistically significant loss in body weight between test and control. All other methodologies identical to ISO.	Necropsy not required. Body weight loss indicative of toxicity if 3 or more lose greater than 10%.	31 days	31 days	4g, 120 cm <sup>2</sup> or 60 cm <sup>2</sup> (x2)	4g, 120 cm <sup>2</sup> or 60 cm <sup>2</sup> (x2)
<b>Subchronic Toxicity (Subacute Toxicity)</b>	Mentions only use of normal saline as extraction vehicle.	NS or CSO can be used.	Only rats can be used. IV dosing for 28 days (90 for subchronic toxicity). Animals must be 5-6 weeks old. Dose of 20 mL/kg.	Mice or rats can be used. Length of study and number of doses not specifically outlined. Daily dosing regimen.	~100 days for 28-Day Subacute	~100 days for 28-Day Subacute	NA	4g, 120 cm <sup>2</sup> or 60 cm <sup>2</sup> (x14)
<b>Pyrogen</b>	Extraction methods identical		Standard methodology identical: 3 rabbits for initial test, 5 rabbits for continued test. Temperature monitoring for three hours post injection of test article.		29 days	29 days	NA	4g, 120 cm <sup>2</sup> or 60 cm <sup>2</sup> (x14)

\* Typical methods. (Other times and temperatures are still in common use.)