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1786 CROSS-VALIDATION OF TWO HOLOGIC-QDR 2000 PLUS DXA IMAGING SYSTEMS FOR IN VIVO EVALUATION OF SOFT TISSUE COMPOSITION.

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Our laboratory employs Dual-energy X-ray Absorptiometry (DXA) in preclinical toxicology studies of anti-obesity drugs in dogs and monkeys. The volume of tissue imaging performed in our laboratory necessitated cross validation of 2 DXA systems in order to use them interchangeably and facilitate scheduling of studies. Both systems had been previously individually validated and therefore the current validation was limited to a verification of reproducibility of soft-tissue measurements between the 2 instruments. To avoid variation resulting from inter and intra-scan differences in basal metabolism of a live animal (*e.g.,* respiratory, digestive and fluid movements), or autolytic changes of an intact, euthanised animal, it was considered inappropriate to employ an animal for the validation. Instead 2 blocks of meat were evaluated; one small (0.8 kg) - and including a removable fat layer; and one large (6 kg) - approximating the scan-size of a beagle dog. For each of the following combinations, 5 or 6 scans were performed on each instrument: 1) Small block with fat layer; 2) Small block without fat layer; 3) Large block. The precision of the repeat measurements within the same unit and between the 2 units was considered acceptable if the coefficient of variation for each parameter (fat mass, lean mass and %fat) was ≤10%. With the exception of the inter-unit comparison of fat mass and %fat of the Small block without the fat layer, the coefficients of variation were ≤9% for all within- and inter-unit comparisons. For the 2 inter-unit comparisons which exceeded the acceptance criteria (C.V.=13% in both instances), it was considered that the failure to meet criteria was due to the small size (approx. 0.7 kg) of the meat block (without the fat layer) and the resulting relatively low amount of fat present. Therefore, it was considered that the units could be used interchangeably, on the condition that the size of the animal to be scanned should be not less than approximately 1 kg.

1787 *IN VITRO* EVALUATION OF BIOCOMPATIBILITY OF A DOSE FORMULATION.

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Captisol® is an anionic beta-cyclodextrin derivative with an average of seven sulfobutyl ether, sodium salt, substituents attached to the parent cyclodextrin. It is used as an enabling excipient for systemic delivery of drugs with poor water solubility, stability or bioavailablitity. The increases in drug solubility obtained with Captisol® allow the development of parenteral products without the requirement for non-physiological pH conditions or the use of mixed co-solvent systems. Propofol is a sedative-hypnotic agent for use in the induction and maintenance of anesthesia or sedation. It is very slightly soluble in water and, thus, is currently formulated and marketed in an oil-in-water emulsion. The biocompatibility of a 22% (w/v) Captisol® with 10 mg/mL propofol in water dose formulation was evaluated for *in vitro* hemolytic, red blood cell (RBC) agglutination, and plasma protein precipitation potential. A commercially available formulation, Diprivan® brand propofol was evaluated as a comparator. Fresh heparinized human plasma or whole blood was combined with each formulation at formulation:whole blood or plasma concentration ratios (v:v) ranging from 0.001:1 to 1:1. Hemolysis was assessed macroscopically and by quantitation of plasma hemoglobin using the cyanmethemoglobin method. RBC agglutination was assessed macroscopically. Protein precipitation in plasma was assessed macroscopically and measured photometrically at 620 nm. The Captisol® formulation showed no indication of hemolytic, RBC agglutination, or plasma protein precipitation potential. Diprivan® brand propofol exhibited slight to moderate hemolytic potential at concentration ratios of 0.1:1 and above. This would indicate that the Captisol® formulation is more biocompatible than Diprivan® under these study conditions.

1788 CYTOPLASMIC VACUOLES IN RENAL TUBULAR EPITHELIUM OF MICE GIVEN POLYETHYLENE GLYCOL (PEG) CONJUGATED PROTEIN ARE REDUCED BY ALTERING PEG SIZE AND CONFORMATION.

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The active half-lives of many biopharmaceutical agents may be extended by conjugation to moieties, such as PEG (J. Pharmaceutical Sci. 83:601, 1994), which delay protein metabolism and/or excretion. Under some conditions, renal clearance of PEGylated proteins results in accumulation of cytoplasmic vacuoles in tubular epithelial cells, without altering renal function (Toxicol. Sci. 42:152, 1998). This study was initiated to assess whether modifications to the PEG side chain could modulate this change. Young adult, female, C57BL/6 mice (n = 5/group) were given daily subcutaneous injections of a PEGylated 16 kD protein or vehicle (phosphate buffered saline; PBS). Kidneys were immersed in neutral buffered zinc formalin, embedded in paraffin, sectioned at 4 µm, stained with HE, and examined at 40x and 200x. The extent of the vacuolar change in the cytoplasm of renal tubular epithelial cells (subcapsular cortex) was graded semi-quantitatively using a five-tiered scale: absent, minimal, mild, moderate, or marked. Vacuolation was marked after daily administration of protein (10 mg/kg) conjugated to a single PEG chain for 7 days. Using the same protein dose and identical schedule, the vacuolar change was reduced markedly (one to three grades) and, in some instances, the incidence was reduced by either increasing the length or branching of the PEG moiety or by attaching additional PEG molecules. These data show that altering the conjugation protocol may diminish the vacuologenic potential of PEGylated proteins.

1789 REDUCTION OF SKIN IRRITATION WITH MAINTAINENCE OF HIGH TRANSDERMAL FLUX DURING THE TRANSDERMAL ADMINISTRATION OF AN IRRITATING DRUG: THE EFFECT OF FORMULATION.

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Many drug candidates with suitable physicochemical properties for transdermal delivery are skin irritants thus precluding their usefulness. This study determined the in vivo skin irritation potential of neat Diphenhydramine (DPH), in rabbits according to the Draize method. The drug was converted from a water soluble hydrochloride salt to the free base with better penetration properties. A mean primary irritation index (PII) of 4.3 (n=4) was observed denoting moderate skin irritation. Bioengineering measurements, namely transepidermal water loss (TEWL), skin blood flow (SBF) were significantly higher at the dosed sites following patch removal. The skin returned to baseline condition in 7-8 days. The irritation potential of different formulations of DPH free base were then assessed. DPH free base in isopropyl myristate (IPM) at concentrations of 70%, 50% and 25% produced PIIs of 3.6, 2.75 and 1.9 respectively. 25% DPH in a 1:1 mixture of IPM and ethanol (EtOH), 7:3 mixture of IPM and EtOH and 5:95 mixture of oleic acid and propylene glycol (OA/PG) had PIIs of 2.1,1.9 and 1.75 respectively. $TEWL_{max}$ and SBF_{max} were also lower for these formulations. The study also assessed the *in vitro* percutaneous penetration of all the formulations through human skin. The flux achieved by the neat DPH free base was 61 µg/cm^2/hr. Slightly lower fluxes were obtained with the formulations containing lower concentrations of DPH. The formulation of 25% DPH in a 5:95 mixture of OA/PG actually had a flux three fold higher than the neat liquid (174 µg/cm^2/hr). The results from these studies thus suggest that formulation strategies can be applied successfully with drugs having high skin irritation potential thus minimising the resulting irritation upon application while maintaining or improving the delivery profiles.

1790 EXPOSURE TO PYRIDOSTIGMINE BROMIDE, DEET, AND PERMETHRIN, ALONE AND IN COMBINATION CAUSES SENSORIMOTOR PERFORMANCE DEFICIT AND CHOLINERGIC ALTERATIONS IN RATS.

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Male Sprague-Dawley rats (200-250 gm.) were treated with DEET (40mg/kg, dermal) or permethrin (0.13 mg/kg, dermal), alone and in combination with PB (1.3mg/kg, oral, last 15 days only) for 45 days. Sensorimotor ability was assessed by beam-walk score, beam-walk time, incline plane performance and fore paw grip on day 30 and 45 following the treatment. Animals treated with PB alone or in combination with DEET and permethrin showed a significant deficit in beam-walk score as well as beam-walk time. All chemicals, alone or in combination resulted in a significant impairment in incline plane testing on day 30 and 45 following treatment. Treatment PB alone caused moderate inhibition in midbrain acetylcholinesterase (AChE) activity. A combination of PB and DEET led to significant decrease in AChE activity in midbrain and brain-

stem. A significant decrease in brainstem AChE activity was observed following combined exposure to PB and permethrin. Co-exposure with PB, DEET and permethrin resulted in significant inhibition in AChE in brainstem and midbrain. Treatment with PB alone caused a significant increase in ligand binding for m2 muscarinic acetylcholine receptor (mAChR) in the cortex. Thus, these results suggest that exposure to physiologically relevant doses of PB, DEET and permethrin, alone or in combination, leads to neurobehavioral deficits and region-specific changes in AChE and mAChR receptor. Supported, in part by the U.S. Army Medical Research and Materiel Command under contract #DAMD 17-99-1-9020. The views, opinion and/or findings contained in this report are those of the authors and should not be construed as an official Department of the Army position, policy or decision unless so designated by other documentation.

1791 HISTOPATHOLOGICAL CHANGES AFTER ORAL ADMINISTRATION OF CYCLOPHOSPHAMIDE (CPA) WITH AND WITHOUT METHOTREXATE (MTX) IN FEMALE ALBINO RATS.

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The effects of daily administration of Cyclophosphamide (CPA) with and without methotrexate (MTX) treatment were studied in several groups of adult albino rats. Post mortem examination was performed 30, 45 and 60 days post administration and specimens from different organs were collected and fixed in 10% buffered neutral formaline solution. Five microns thickness paraffin sections were prepared and stained by hematoxylin and eosin stain and the histopathological changes were investigated. The lesions in CPA and MTX were nearly similar and increased with the increase of dose and duration. CPA induced damage in the vascular beds, resulting in hemorrhages in different organs beside severe depletion of lymphoid tissues from lymphocytes. Thrombosis, coagulative necrosis, lymphocytic infiltration and hepatocellular carcinoma were observed. The carcinoma was represented by multifocal nodules of hepatocytes with pleomorphism with atpical mitosis. MTX administration produced hepatocyte swelling, fatty change and necrosis. Co-administration of both chemicals ameliorated the pathological changes. The findings from this study may suggest that the co-administration Of CPA and MTX may reduce the histotoxic of each individual drug.

1792 EFFECT OF DAPD ON HUMAN DNA POLYMERASES AND MITOCHONDRIAL FUNCTIONS.

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(-)-β-D-2,6-diaminopurine dioxolane (DAPD), the prodrug of the nucleoside reverse transcriptase inhibitor (-)-β-D-dioxolane guanosine (DXG), is active against HIV-1 and HBV, *in vitro* and *in vivo*. Adenosine deaminase is the enzyme responsible for converting DAPD to DXG. Subsequently, DXG is metabolized to the 5′-triphosphate, which is a potent inhibitor of HIV-1 (EC_{50}= 0.105μM). Antiviral nucleoside therapy can produce side effects in treated patients, but the mechanisms of toxicity are poorly understood. One theory for nucleoside toxicity is that the nucleoside analog also acts as a substrate for host DNA polymerases, interrupting host DNA replication. In this study, DXG 5′-triphosphate (DXG-TP) has been evaluated biochemically for activity against the host DNA polymerases. Human DNA polymerase α and human DNA polymerase β do not utilize DXG-TP efficiently as a substrate under *in vitro* steady state conditions. Steady state kinetic analysis determined that DXG-TP is a competitive inhibitor of pol γ(pol γ), however an insubstantial $K_i/K_{m\text{-}dGTP}$ ratio of 1.4 was measured. Additionally, pre-steady state kinetic analysis determined that DXG-TP is incorporated 113-fold less efficiently than dGTP during DNA synthesis catalyzed by pol γ. These results explain the lack of mitochondrial toxicity observed in a mitochondrial toxicity tissue culture assay. Neither DAPD nor DXG produced measurable effects on mtDNA synthesis, lactic acid production, and overall cell viability in tissue cultures treated with DAPD or DXG at clinically relevant concentrations. Long term safety studies of DAPD for the treatment of HIV-1 and HBV are ongoing.

1793 THE USE OF FLOW CYTOMETRY TO EVALUATE BONE MARROW TOXICITY IN THE RAT.

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Effects on the bone marrow are a common toxicity in some classes of drugs. The assessment of drug toxicity in bone marrow has traditionally relied on time-consuming processes such as cell counts, gross and histopathology of bone marrow. The purpose of this study was to investigate the potential of flow cytometry to differentiate between the different cell lineages in the bone marrow. Several different flow cytometry methods were developed in this study to characterize normal rat bone marrow. The different cell types were analyzed based on their capacity to alter the forward scatter (FS) and side scatter (SS) of light. Cells from 8 to 10 regions were sorted on an Epics Cell Sorter and were evaluated histologically. The position of the entire maturation sequence for RBCs, lymphocytes, granulocytes, and monocytes could be documented on the FS *vs.* SS scattergram. Some populations containing mixed cell populations could be further differentiated by qualitative and quantitative immunophenotyping techniques. CD 45R-PE and CD71-FITC double staining allowed specific quantitation of the B-lymphocytes and transferrin receptors found on maturing erythrocytes in the regions where they overlapped. The cell cycle of G0-G1, S and G2-M phases was analyzed using propidium iodide, which intercalated into DNA. Most published methods use detergents that disrupt the cell membranes and not allow differential evaluation of specific cell types. We developed a method that both preserved the morphology of the cells and allowed propidium iodide and Rnase to enter the nucleus, which allowed cell cycle analysis on different cell lineages to be possible. This method used 0.5% paraformaldehyde to fix the cells and 1% digitonin to permeablize them. Mitochondrial membrane potential and function could be evaluated using nonyl acridine orange and rhodamine 123, respectively. In conclusion, flow cytometry methods aid more understanding of pathogenesis over the traditional methods in evaluating drug-mediated bone marrow effects.

1794 CARDIOVASCULAR EFFECTS OF AMLODIPINE COMBINED WITH HALOTHANE OR ISOFLURANE.

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Severe cardiovascular adverse events have been reported in patients on calcium blockers, in particular on verapamil and dilthiazem, when administered halogenated anesthetics. In order to test whether similar effects can be expected with the dihydropyridine calcium blocker amlodipine (Am), the combined effects of Am and halothane (Ha) or isoflurane (Is) were studied blindly in 24 closed-chest dogs given 0.1 mg/kg Am or a placebo, and Ha or Is at 1.2 MAC 60 min later. The decrease in sinus rate (-8%), the increase in auriculoventricular conduction time (+34%) and the prolongation of atrial effective refractory period (ERP) (+32%) were not significantly more marked with Am when combined with Ha than with the placebo. Significant changes of halothane effects were observed on mean blood pressure (-29%) and left ventricular (LV) dP/dt max (-35%) at Am plasma concentrations ranging between 5.2 and 3.5 ng/ml. Sinus rate and auriculoventricular conduction time remained unchanged with the combination amlodipine and isoflurane. In contrast, atrial ERP was more prolonged. Likewise, amlodipine at plasma concentrations between 8.4 and 3.9 ng/ml potentiated the reduction in mean blood pressure (-26%) and LV dP/dt max (-34%) caused by isoflurane. Based on our experimental data, the potentiation of the depressant cardiovascular effects of amlodipine by halothane or isoflurane is considered too weak to justify contraindication of these anesthetics in patients treated with amlodipine.

1795 ACUTE CARDIOVASCULAR EFFECTS OF ROPIVACAINE AND BUPIVACAINE IN ANESTHETIZED PIGS PRETREATED WITH IA OR IC ANTIARRHYTHMIC DRUGS.

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Bupivacaine is the most cardiotoxic amide local anesthetic, the cardiovascular effects of which are potentiated by class IA and IC antiarrhythmics. Ropivacaine, a more recent derivative, is considered less cardiotoxic. The aim of this study was to compare the cardiovascular effects of bupivacaine and ropivacaine when combined