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Establishing maximum tolerated doses for a 2-year combined chronic/carcinogenicity rat study based on toxicokinetic and toxicity gender differences

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ABSTRACT

For agrochemicals tested in a carcinogenicity rodent study, it is often not possible to use the same high dose to achieve maximum tolerated dose (MTDs) without overdosing or insufficiently challenging one gender if significant gender differences are known. Toxicokinetic (TK) data for pesticide FR from a 28-day rat study showed that males required a 3-fold higher external dose compared to females to produce similar internal exposure levels of the parent compound. In the 90-day study, 8%/17% (M/F) decrease in bodyweight gain (BWG) and 15%/15% (M/F) increase in relative liver weights were observed in the 6000 ppm males and 2000 ppm females, respectively. Based on the above TK and toxicity data, different high dose levels were selected for females (1600 ppm) and males (4800 ppm) for a 2-year combined chronic/carcinogenicity study in rats. In the 2-year study, 14%, 13%, 13% and 21% reduction in BWG of males and 10%, 12%, 19% and 20% reduction in BWG of females were observed at weeks 13, 26, 52 and 104, respectively in the highest dose tested. Similar reductions in bodyweight gain in males and females at the different high dose levels clearly demonstrated that appropriate MTDs were reached. Therefore, it is scientifically sound and practical to use TK and toxicity data to use different high dose levels to achieve MTDs for a pesticide with large gender differences.

1. Introduction

Agrochemicals are required to be tested up to the maximum tolerated dose (MTD) in carcinogenicity studies in rats and mice for regulatory approval. Failure to achieve the MTD or observe toxicity at the highest dose level in a carcinogenicity study may compromise the hazard identification of the carcinogenicity activity of the test substance and may lead to a rejection or partial acceptance of data as supplemental information. Therefore, dose selection for a chronic and/or carcinogenicity study requires careful evaluation of the accumulated toxicokinetic (TK), absorption, distribution, metabolism and excretion (ADME) and toxicity data on the test substance. Additional evaluation to select dose levels may also include read-across from compounds which share similar relevant structure, physical-chemical, mechanistic or metabolic activity (Farber, 1980; Rhomberg et al., 2007).

OECD 453 and US EPA OPPTS 870.4300 test guidelines require that the highest dose level should elicit minimal signs of toxicity without shorten the normal life span due to effects other than neoplastic development in a carcinogenicity study. In general, in the absence of any other signs of toxicity (e.g., organ weight, clinically significant changes in hematologic, urinary, clinical chemistry and more definitive toxic and histopathologic endpoints), a 10% decrease in body weight gain relative to controls over the duration of a study in young adult animals of at least 90 days provides evidences that an adequate high dose or MTD is reached in a carcinogenicity study. Over the last two decades, new criteria that have evolved for the selection of an adequate high dose also include: kinetically-derived maximum dose (KMD) considering

Abbreviations: ADME, absorption, distribution, metabolism, and excretion; AUCs, areas under the curve; C_{max}, maximum concentration; KMD, kinetically-derived maximum dose; MTD, maximum tolerated dose; TK, toxicokinetic; T_{max}, time to reach maximum concentration.

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toxicokinetics endpoints, saturation of absorption, metabolism or excretion; pharmacodynamic endpoints; maximum feasible dose (MFD) or a limited dose of 1000 mg/kg for a repeated-dose study (Rhomberg et al., 2007; Saghir et al., 2012). For pharmaceutical agents, high dose selection is generally based on attaining high multiples of the maximum recommended human daily dose (>100X on a mg/kg basis) or a high dose representing a 25-fold higher exposure in rodents compared to human plasma AUC of parent compound (US Department of Health and Human Services, 2008; OECD, 2012).

Predicting and then selecting an accurate MTD for a carcinogenicity study based solely on sub-chronic toxicities observed in range-finding studies is often a challenge. For a chemical with significant gender differences in toxicokinetics and toxicity, it is often virtually impossible to use the same high dose to achieve MTDs without overdosing the more sensitive gender or insufficiently challenging the less sensitive gender. If the same high dose is chosen for both genders, a carcinogenicity study could be potentially rejected for not attaining MTD in the less sensitive gender or be compromised by unexpected effects (e.g., excessive high mortality which lead to early termination of the sensitive gender groups). The carcinogenicity assessment could be compromised and improper evaluation and hazard classification could result in misplacement of research & development resources, time and animal welfare.

In this paper, we describe the data and methods we used to select doses for a 2-year rat study with a new pesticide active ingredient FR that showed significant gender differences in toxicokinetic and toxicity. Toxicokinetic and toxicity data from sub-chronic toxicity studies, along with the TK data from the ADME studies were used to predict and select different high dose levels to achieve MTDs in male and female rats for a 2-year combined chronic/carcinogenicity study. The dose levels selected for the 2-year rat study included an additional male high dose group and the results showed that MTDs were achieved in both genders with a 3-fold different high dose level between males and females.

2. Materials and methods

2.1. Chemicals

A pesticide active ingredient, FR (Molecular weight ~350, Log Kow ~4 and 97.46% purity), was supplied by FMC Corporation (Ewing, NJ, USA). The purity of the test substance and dosing formulations were tested at FMC and contract research organizations and found to be consistent for the purity and stable during testing.

2.2. Animals and housing

2.2.1. Toxicokinetic study

Toxicokinetic studies were conducted at Accelera Srl, Nerviano, Italy. Male and female (nulliparous and non–pregnant) albino Sprague Dawley (SD) rats (Charles River, Calco, Lecco, Italy) were used in the TK study. Body weights at the time of first dosing were in the range of 250–340 g (male rats) and 214–291 g (female rats). All animals were visually inspected for signs of illness and were deemed fit for use in the study. During the acclimation period (3 days) the rats were group housed in polypropylene and stainless steel cages with wood shavings as bedding (Lignocel, Germany). In the experimental period, the rats were individually housed in polypropylene and stainless steel cages with raised wire mesh floors or in glass metabolism cages. Holding and study areas had automatic control of light cycle and temperature. The lighting was controlled in a 12-h light-dark cycle throughout the study. Temperature and relative humidity measured during the study were in the range of 20–23 °C and 40%–70%, respectively. Diet and water were available *ad libitum* throughout the study. The animals were fasted overnight before each dosing with radiolabeled test item; on the day of dosing food was offered about 4 h post dosing. The animals were not fasted for the repeat dose study.

2.2.2. Toxicity studies

Male and female SD rats were received from Charles River Laboratories, Inc. (Raleigh, NC, USA). The toxicity studies were conducted at Charles River Laboratory (Ashland, OH, USA) in compliance with the GLP with protocols approved by the Institute of Animal Care and Use Committee. The animal facilities at Charles River Laboratory are accredited by AAALAC International.

All animals were housed for a 14-day acclimation period prior to the start of treatment. In the 28-day and 90-day studies, all animals were housed individually in clean, stainless steel, wire-mesh cages suspended above cage-board during acclimation, following randomization and during the exposure period. In the combined chronic/carcinogenicity 2-year rat study, all animals were pair-housed by sex (2/cage) during acclimation, following randomization and during the exposure period. Animals were housed in clean, solid-bottom cages with nesting material in the 2-year rat study. PMI Nutrition International, LLC Certified Rodent LabDiet® 5002 (meal) was used for administration to the control group and in preparation of the test diets. Enrichment devices were provided to all animals as appropriate throughout the study for environmental enrichment and to aid in maintaining the animals' oral health, and were sanitized weekly.

2.3. Experimental methods

2.3.1. Toxicokinetics study

Plasma toxicokinetics were determined after a single oral (PO) administration of [^{14}C]-labeled FR at doses of 50 mg/kg and 1000 mg/kg, or after a 13-day repeated PO administration of unlabeled FR followed by a last administration of [^{14}C]-labeled FR at a dose of 50 mg/kg. The rats used for TK evaluation were surgically prepared at Accelara, Italy by *superior vena cava* catheterization (SVC) to allow serial blood sample collection; the radioactivity in plasma as well as the parent FR was determined up to 96 h after administration. The compound was administered by gastric gavage at the target doses dissolved in the vehicle (CMC low viscosity 0.5%/Tween 80 0.1%, v/v). Serial blood samples were collected from each animal at 0, 0.25, 0.5, 1, 2, 4, 6, 8, 24, 48, 72 and 96 h points post-dose. The blood samples were centrifuged at 10,000 RCF for 3 min (for the highest blood volumes, at 1500 RCF for 20 min) at +4 °C to collect plasma (within 30 min from blood collection) and single aliquots of plasma were retained for radioanalysis. Radioactivity in test formulation and in the samples was measured by LSC using Packard liquid scintillation analyzers. Samples were counted up to 1 h (with the 2 sigma% settled at 0.30 region A and 0.50 region B), together with representative blank vials. Concentrations of parent FR in plasma were determined by a validated LC-MS/MS method.

2.3.2. Toxicity studies

In the 28-day dietary toxicity study (OECD Guideline 407, US EPA OPPTS 870.3050), FR (0 [control], 300, 1000, 2000, and 4000 ppm) was offered in the diet *ad libitum* for 28 consecutive days to toxicology groups and for 26 consecutive days to TK satellite groups of SD rats. Each toxicology group consisted of 5 animals/sex; each TK group consisted of 3 animals/sex. For toxicokinetic evaluation, blood samples were collected on study days 2 and 24 from 3 animals/sex (control) at approximately 6:00 a.m. and from 3 animals/sex/group at approximately 6:00 a.m., 10:00 a.m., 2:00 p.m.,