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Toxicological Assessment of 2-Methyltetrahydrofuran and Cyclopentyl Methyl Ether in Support of Their Use in Pharmaceutical Chemical Process Development

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ABSTRACT: Herein we document our evaluation of the oral toxicity of MeTHF and CPME as determined in three month repeat-dose toxicity studies in rats as well as a battery of tests conducted under Good Laboratory Practice (GLP) to assess induction of micronuclei, microbial mutagenicity, and chromosomal aberrations. Based on the studies performed, human permitted daily exposure limits of 6.2 and 7.4 mg/day for MeTHF and CPME respectively have been established, with both of these solvents also considered negative for genotoxicity and mutagenicity. In addition, for future standard repeat-dose GLP animal studies a general limit of 20 mg/kg/day and a maximum concentration of 2% of MeTHF or CPME would not be expected to contribute to any toxicity potentially exhibited by an active pharmaceutical ingredient containing these solvents. By sharing these data, we hope to facilitate the use of these ethereal solvents within the pharmaceutical chemical process development community and contribute to the path to their potential ICH classification.

INTRODUCTION

With the pressures facing the pharmaceutical industry to increase the value proposition of new therapeutic agents for both patients and payers, combined with the growth of the emerging markets, the pursuit of green chemistry principles[1−3] as a means to reduce the cost basis, sustainability, and carbon footprint of the synthesis of drug substances has never been more relevant. The design of chemical processes that reduce or eliminate the use of hazardous materials and minimise the associated waste production is critical to this endeavor. The introduction of new synthetic technologies and their associated chemical reagents and solvents engenders potential challenges within the strict regulatory environment with which those engaged in drug substance preparation must operate. In particular, control of the residual levels of any impurities per ICH guidance Q3A(R2)[4] and specifically of residual solvents per guidance Q3C(R4)[5] is required.

The solvents 2-methyltetrahydrofuran[6] (MeTHF: CAS 96-47-9) and cyclopentyl methyl ether[7] (CPME: CAS 5614-37-9) (Figure 1) are being increasingly used[8,9] within the academic and industrial chemical communities as alternatives to their more common analogues such as tetrahydrofuran (THF: CAS 109-99-9) and *tert*-butyl methyl ether (TBME: CAS 1634-04-4). They offer superior physical (water azeotrope, phase cuts, lower volatility) and chemical properties (greater acid/base stability, higher flash point) as well as being commercially available at scale.[10] Moreover, MeTHF is derived from renewable sources through the catalytic reduction of furfural which is itself available by dehydration of C-5 sugars present in biomass.[11] For these reasons MeTHF and CPME are generally considered as a greener alternative to THF, and their use is advocated by the ACS Green Chemistry Pharmaceutical Roundtable.[12]

Whilst a nomination to the U.S. National Toxicology Program (NTP) to study MeTHF was made by the National Cancer Institute due to the increased usage of this material as an alternative fuel, to date only limited toxicological information has been disclosed. In particular, an ADME study evaluating both oral and intravenous administration of [^{14}C]-MeTHF has been reported with no overt signs of toxicity at any dose studied.[13] Ames (*Salmonella typhimurium*) and L5178Y lymphoma mutagenicity evaluations have also been reported.[14] Significant toxicological studies of CPME that establish acceptable human exposures have not been reported within the published literature. Currently, neither MeTHF nor CPME is classified or has recommended human permitted daily exposure (PDE) within ICH Q3C(R4)[5] due to the lack of available data and as such could be considered to fall under ICH Q3A(R2). This raises an additional hurdle to their use in the late stages of a chemical synthesis where these solvents could potentially persist in the drug substance that is used for exploratory preclinical safety assessment and human clinical studies.

In this article we describe our evaluation of the oral toxicity of MeTHF and CPME as determined in three month repeat-dose toxicity studies in rats as well as a battery of tests to assess induction of micronuclei, microbial mutagenicity, and chromosomal aberrations conducted under Good Laboratory Practice (GLP). By sharing these data, our intent is to facilitate the

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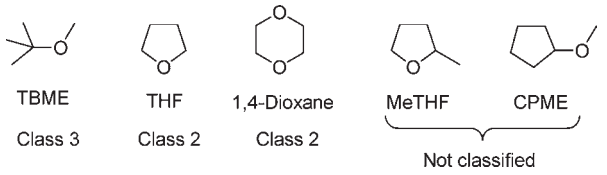

Figure 1. Selected ethereal solvents and their respective ICH Q3C(R4) classifications.[5]

increased usage of these greener solvents within the chemical process development community and beyond and support the path to ICH classification of these solvents.

RESULTS AND DISCUSSION

Mutagenicity Studies. The mutagenicity of MeTHF and CPME were evaluated in the Ames test using *Salmonella typhimurium* strains TA1535, TA1537, TA98, TA100 and *Escherichia coli* strain WP2 *uvrA*. The plate incorporation version of the bacterial mutation test was performed, with and without a supplemented liver fraction (S9 mix) for metabolic activation. Due to the volatility of MeTHF and CPME, plates containing these materials were sealed in Tedlar gastight bags during incubation. Bacteria were incubated with standard positive control agents, and the response of the various bacterial strains to these agents confirmed the sensitivity of the test system and the activity of the S9 mix. No substantial increases in the revertant colony counts were obtained with any strain following exposure to MeTHF up to 5490 μg/plate or CPME up to 5710 μg/plate in either the presence or absence of the S9 mix, and there was no apparent toxicity to the bacteria. Seifried et al.[14] have previously shown that MeTHF is negative in both the Ames *Salmonella typhimurium* and L5178Y mouse lymphoma cell mutation assays at doses up to 10 000 μg/plate and 5000 μg/mL respectively. From these data, we conclude that these two solvents do not show any evidence of mutagenic activity in *in vitro* tests tested in accordance with regulatory guidelines.[15]

Chromosome Damage Studies. The genotoxicity of MeTHF and of CPME was assessed using an *in vitro* chromosomal aberration test. Human peripheral blood lymphocytes were stimulated into division in culture and then treated with MeTHF or CPME at a range of concentrations up to a target of 10 mM, the standard limit dose for this test. Due to the volatile nature of the test articles, the treatment tubes were sealed during incubation. Cultures were treated for 4 h with or without S-9 metabolic activation and for 21 h without S9. There was no evidence of toxicity (mitotic suppression) up to 10.7 mM MeTHF or 11.3 mM CPME and no statistically significant increases in the proportion of cells with chromosome aberrations. Positive controls Mitomycin C and cyclophosphamide confirmed the sensitivity of the system and the effectiveness of the S9 mix. We conclude that MeTHF and CPME do not show any evidence of genotoxic activity in this *in vitro* test for induction of chromosome damage when tested in accordance with regulatory guidelines.[15] In addition, a micronucleus assay was conducted on bone marrow cells from the three-month rat study. Slides were prepared from bone marrow cells that were harvested at sacrifice the day after the canal dose and were stained with acridine orange. Positive control slides from male rats previously treated with mitomycin C were included in the coded slides scored. Two thousand polychromatic erythrocytes (PCE) per rat were scored for micronuclei (MN-PCE) from coded slides from each of 10 vehicle-treated rats per sex for the vehicle and negative control groups, each of 5 low- and high-dose positive control male rats, and from each of 5 test article-treated rats per group per sex. The frequencies of PCE and of mature, normochromatic erythrocytes (NCE) were also recorded among 1000 erythrocytes per rat. Overall the study was negative with no marked effect on the proportions of bone marrow PCE among total erythrocytes (NCE) in the bone marrow.

Three Month Repeat-Dose Oral Toxicity Studies. The potential toxicity of MeTHF and of CPME was evaluated when administered separately to male and female Sprague−Dawley Crl:CD(SD) rats by oral gavage daily for approximately three months. The top doses tested were 26 mg/kg/day for MeTHF and 31 mg/kg/day for CPME using corn oil as the vehicle.[16] The assessment of toxicity was based on mortality, clinical observations, body weights, food consumption, opthalmic examinations, and histopathology evaluations. There were no test article-related antemortem or postmortem (organ weight, gross, or histomorphologic) findings with either test article at the top dose tested. The exposures in the animal studies provide human PDE values of 6.2 and 7.4 mg/day for MeTHF and CPME respectively based on a 60 kg individual and using extrapolation factors of 5 (rats to humans), 10 (individual variability), and 5 (animal exposure duration).[17] For future standard repeat-dose GLP animal studies a general limit of 20 mg/kg/day and a maximum concentration of 2% of MeTHF or CPME would not be expected to contribute to any toxicity potentially exhibited by an active pharmaceutical ingredient containing these solvents.

CONCLUSIONS

Based on the studies performed, qualification and human permitted daily exposure limits for MeTHF and CPME have been established, with both of these solvents also considered negative for genotoxicity and mutagenicity. Taken together these new data should assist in broadening the usage of these solvents in early phase development where the drug substance is being prepared for short-term safety and clinical studies since low levels would not be expected to contribute to any toxicity potentially exhibited by the drug substance under investigation. In addition, these data contribute to the potential path to formal ICH classification for these greener ethereal solvents and we hope that others within the chemical and pharmaceutical community will participate in this endeavor.

AUTHOR INFORMATION

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