optimization phase with a small number of candidates. The species selection is based on the same principles as the selection of the species for the pivotal studies. There are no fixed rules on animal number, dose selection, study parameter selection, and treatment duration. In addition, whether only a rodent species or a rodent and a non-rodent species are used for screening may be decided case by base based on prior knowledge on the compound class, the pharmacological mode of action, and the level of concern related to the individual drug candidate. Since the pilot repeat-dose studies may also be used as dose-range-finding studies for the pivotal studies, testing in the species also intended for the pivotal repeat-dose toxicity studies for FiM at dose levels supporting dose rationale of the pivotal studies helps limiting the number of animals used for the testing program to a minimum. Sometimes, availability of test substance may be a factor to be taken into account, when planning the studies.

These pilot studies are mostly performed as short-term repeat-dose toxicity studies with treatment duration of 3–14 days. Analysis of larger data sets of small-molecule drugs showed that 14-day toxicity studies in a rodent and a non-rodent species are best suited to reduce attrition rate during GLP phase (Roberts et al. 2014). However, there are still relevant adverse effects only found after longer-term treatment with most being detected in the 4-week repeat-dose toxicity studies (Olsen et al. 2000; Greaves et al. 2004; Tamaki et al. 2013), so that studies with 4 weeks of dosing are the best approach for the pivotal GLP toxicity studies enabling first-in-man studies, although in principle, a 2-week duration would be sufficient according to minimum standards defined in ICH M3 (R2).

When using the pilot repeat-dose toxicity studies also as dose-range-finding studies for the pivotal GLP studies, the high dose should be selected based on the principles described in ICH M3 (R2). Criteria are the reaching of the maximum tolerated dose (MTD) or the maximum feasible dose (MFD), e.g., characterized by exposure saturation, testing of a limit dose (generally 1,000 mg/kg for repeat-dose), or testing of a high multiple of the anticipated human dose (50 times the intended human therapeutic dose) (Buckley and Dorato 2009). For definition of MTD, body weight development may be used in addition to clinical signs (Chapman et al. 2013). Margins of exposure for general toxicity studies may be estimated based on the human equivalent dose (HED) as defined by the FDA guidance for industry (2005) or based on fraction unbound of the AUC.[9]

Study design in these studies should be tailored to the compound. The parameters tested depend on the endpoints to be covered. Histopathology should at least include the vital organs like the heart, lungs, liver, kidneys, and brain, some endocrine organs, some lymphatic tissue, and the administration site (e.g., gastro-intestinal tract for oral dosing and veins for i.v. dosing). Animal number in rodents should be high enough to allow adequate sampling for toxicokinetics evaluation. In non-rodents, 1 to 2 animals per group may be sufficient. In cases, where the

[9] Area under the curve