**Supplementary Table 3: Blood plasma concentration at LOED *in vivo* higher than LOEC *in vitro***

**A: Data from *in vitro* micronucleus (MNvit) studies**

| **No.** | **Chemical (therapeutic area or proposed use)** | **LOEC for MNvit (µg/mL); treatment conditions; aneugen or clastogen (if determined)** | **Concentrations scored in MNvit (µg/mL).**  **Other concentrations, treatment times, conditions scored** | ***In vitro* cell type used**  **(e.g. human lymphocytes [HLC], TK6, V79, CHO etc.)** | **Dose levels scored for *in vivo* MN**  **(mg/kg); number of administrations** | **Lowest +ve dose *in vivo* (LOED)** | **Endpoint (species, tissue) giving LOED** | **Blood or plasma conc. at LOED (at doses used in study or extrapolated from other doses/studies)** | **Ratio of plasma LOED:LOEC** | **Comments** |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| 1 | Antifolate for Oncology | 0.002 µg/mL  (24+24 hr –S9);  no mechanistic follow up | 0.001, 0.002, 0.004, 0.016 µg/mL  (24+24 hr -S9)  51% cytotox. at 0.016 µg/mL | HLC | 5, 50 and 200 (estimated MTD) mg/kg/day  (2 admins, 0 and 24 hrs; i.v. dosing) | 5 mg/kg/day  LOED may be lower since +ve at all doses tested | MN (rat bone marrow) | ≤5.260 or 5.680 µg/mL (2 analytes)  Data collected from doses in study | >2630  (5.25/0.002) | LOED not identified since strong *in vivo* MN response at all dose levels |
| Negative at 3+21 hr +/-S9 at cytotoxic concs. | * 0.005, 0.010, 0.020 µg/mL (3+21 hr –S9)   47% cytotox. at 0.02 µg/mL   * 0.01, 0.02, 0.04, 0.2 µg/mL (3+21 hr +S9)   52% cytotox. at 0.2 µg/mL |
| 2 | Compound A  (Oncology) | 6 µg/mL (24+0 hr –S9); aneugen | 4, 6, 8 µg/mL  (24+0 hr -S9) | TK6 cells | 10, 30, 100 and 300 mg/kg/day  (2 admins, 0 and 24 hrs) | 100 mg/kg/day | MN (rat bone marrow) | 26 µg/mL  (measured at doses used in the study) | 4.33  (26/6) | ANEUGEN by *in vivo* FISH analysis |
| Also +ve at 200 µg/mL (3+24 hr –S9) and 135 µg/mL (3+24 hr +S9) | * 200, 250, 300 µg/mL   (3+24 hr -S9)   * 135, 150, 165 µg/mL   (3+24 hr +S9) |
| 3 | Compound C  (CNS disease) | 3.5 µg/mL  (24+0 hr -S9);  39.3 µg/mL  (6+18 hr -S9). Likely to be aneugen | * 2.3, 3.5, 5.3, 7.9, 11.9 µg/mL (24+0 hr, –S9) * 39.3, 49.2, 61.4 µg/mL (6+18 hr, -S9) | CHL cells | 500, 1000 and 2000 (limit dose) mg/kg/day (2 administrations, 0 and 24 hrs) | 500 mg/kg/day | MN (rat bone marrow)  No other endpoints or tissues studied | 57.5 µg/mL  (extrapolated from plasma conc. after a single dose of 100 mg/kg) | 16.4  (57.5/3.5) | Also positive for polyploidy in CAvit at similar concentration  PROBABLE ANEUGEN |
| 5.6 µg/mL  (24+0 hr -S9).  Negative in the other treatment conditions | * 5.6, 7.28 µg/mL (24+0 hr, –S9) * 50, 60 µg/mL   (3+21 hr, -S9)   * 41, 51.2 µg/mL   (3+21 hr, +S9) | TK6 cells |
| 5 µg/mL  (24+0 hr -S9).  Negative in the other treatment conditions | * 4, 5, 6 µg/mL (24+0 hr, –S9) * 20, 24 µg/mL   (3+21 hr, -S9)   * 31, 38 µg/mL   (3+21 hr, +S9) | HLC |
| 4 | Compound E  (CNS disease) | 29.4 µg/mL  (24+0 hr -S9). | 19.6, 29.4, 44.1, 66.2, 99.3 µg/mL (24+0 hr, –S9) | TK6 cells | 50, 200, 500, and 2000 (limit dose) mg/kg/day  (3 admins, Comet-MNT combination, examined 4 hrs after last dose) | 2000 mg/kg/day | MN (rat  bone marrow)  Negative comet in stomach and liver | 280 µg/mL  (extrapolated from plasma conc. at 300 mg/kg) | 9.52  (280/29.4) |  |
| Negative in the other treatment conditions | 19.6, 29.4, 44.1, 66.2, 99.3, 148.9, 223.3, 335 µg/mL (3+21 hr, +/-S9) |
| 5 | #1 Drug for cancer | <500 ng/mL (lowest conc. tested; 4+20 hr -S9) | 500, 600, 700, 800, 900, 1000 ng/mL  (4+20 hr -S9) | L5178Y (Mononucleate cells) | 0.25, 0.84, 2.5 (MTD) mg/kg/day  (2 IV admins,  0 and 24 hrs) | 2.5 mg/kg/day | MN (rat bone marrow)  No other endpoints or tissues studied | 919.1 ng/mL  (from satellite animals in MN study, collected 5 min post-dose Day 1) | >367  (919.1/2.5) | Positive at high dose only (5.9-fold control)  ANEUGEN |
| 2.5 ng/mL (24+12 hr -S9);  Aneugen | 0.1, 0.5, 1.0, 2.5, 5, 10 ng/mL (24+12 hr – S9) |
| 6 | #7 Drug for cancer (same class as #6 Drug for cancer – see Supplementary Table 2) | No clear +ve responses 3+21 hr +/-S9 up to ICH limit. | 200, 400, 500 µg/mL (3+21hr +/-S9) | HLC | 30, 375, 750, 1500 mg/kg/day (MTD)  (3 PO admins,  0, 24 and 47.5hrs)  Combined MN/comet. | 375 mg/kg/day  (1.5-fold increase but stat. sig.) | MN (rat bone marrow).  Liver Comet Negative | 6.77 µg/mL (prodrug), 25.8 µg/mL (active metabolite) from satellite animals in MN/Comet study, collected at Tmax (0.5hr) on Day 1) | >2.26  (6.77/3) | Max 2.2-fold control at 1500mg/kg/day  ANEUGEN |
| Inconclusive at 3 µg/mL 24+24 hr -S9 | 0.25, 1,2, 3 µg/mL (cytotoxic; 24+24hr -S9) |
| <4µg/mL (24+0 hr -S9) since +ve at all concs. tested; aneugen | 4, 16, 25, 50 µg/mL (limited by toxicity 24+0hr -S9) |
| 7 | #9 Drug for Parkinson’s | <2.5µg/mL (24+24 hr -S9) since +ve at lowest conc. tested;  aneugen | 2.5, 3.5, 4µg/mL (limited by toxicity 24+24hr -S9) | L5178Y  (Mononucleate cells) | 125, 250, 500 mg/kg/day  (3 oral admins,  0, 24 and 44 hrs; combined MN-comet) | 125 mg/kg/day but equivocal at all doses (stat. sig. increases, shift in distribution, but within HCR);  Max. 2.4-fold at 250 mg/kg/day | MN (rat bone marrow).  Rat liver Comet –LOED 250 mg/kg/day but may be confounded by liver toxicity - minimal focal haemorrhage/necrosis seen at 125 & 500mg/kg/day, adaptive changes (minimal hypertrophy at 250 & 500 mg/kg/day, minimal-slight reduced hepatocyte glycogen at all doses) | 18.7 µg/mL extrapolated from plasma conc. at 500 mg/kg (satellite animals in MN/Comet study) | >7.48  (18.7/2.5) | No plasma sampling of lower doses  ANEUGEN |
| 75 µg/mL (3+21 hr +S9); | 20, 60, 75 µg/mL (cytotoxic; 3+21 hr +S9) | 2nd MN study:  1, 3, 10, 30, 125 mg/kg/day  (2 oral admins, 0 and 24 hrs) | 2nd MN study:  equivocal at 125 mg/kg/day | 2nd MN study: 60.7 μg/mL at 125 mg/kg/day | 2nd MN study: Negative at 1, 3,10, 30 mg/kg/day |
| 90 µg/mL (3+21 hr -S9) | 2.5, 40, 80, 90 µg/mL (cytotoxic; 3+21hr – S9) |  |  |  |  |
| 8 | #10 Drug for Parkinson’s (same class as #9 Drug for Parkinson’s – see above, and #11 Drug for Parkinson’s – see Supplementary Table 1) | <2 µg/mL (24+24 hr -S9) since +ve at lowest conc. tested;  aneugen | 2, 4, 10, 40 µg/mL (cytotoxic; 24+24hr -S9) | L5178Y  (Mononucleate cells) | 1-month tox study with integrated MN; 5, 15, 50 mg/kg BID, i.e. 28 total daily doses 10, 30, 100 (MTD) mg/kg/day) | 100 mg/kg/day for MN in  1-month tox study | MN (mouse bone marrow);  (decreases in hematopoietic cells in femur/sternum bone marrow in all animals given 100 mg/kg/day) | 6.84 µg/mL (males), 11.9 µg/mL (females) at 100 mg/kg/day on Day 28 | >3.42  (6.84/2) | ANEUGEN |
| <20 µg/mL (3+21 hr -S9) since +ve at lowest conc. tested | 20, 100, 140 µg/mL (cytotoxic; 3+21 hr-S9) | Combined MN/comet 150, 300, 600 (MTD) mg/kg/day  (3 oral admins, 0, 24, 47.5 hours) | Comet LOED 150 mg/kg/day stat. sig. increases but assoc with liver tox - hepatocyte degeneration/necrosis, elevated ALT, AST, GLDH (all groups) | Mouse liver Comet– may be confounded by liver toxicity –  MN endpoint not scored due to poor cell morphology | Mean plasma 18.3 µg/mL at 150 mg/kg (satellite animals in MN/Comet study) | Stat. sig. increases in comets also observed at 600 mg/kg/day (not sig. at 300 mg/kg/day) |
| 100 µg/mL (3+21 hr +S9) | 40, 100, 120 µg/mL (cytotoxic; 3+21 hr +S9) |
| 9 | BMS-1 (a microtubule stabilizer) | 5.01 ng/mL (22+0 hr -S9)  Aneugen | 0.627, 1.25, 2.51, 5.01 ng/mL (22+0 hr –S9)  42% cytotox. at 5.01 ng/mL | CHO-WBL cells | 0.1, 0.3, and 1.0 mg/kg (iv, once weekly for 9 wks)  Bone marrow sampled Day 58 (~24 hrs after 9th injection) | 1.0 mg/kg in males  Negative in females (same doses) | MN (rat bone marrow)  No other endpoints or tissues studied | 68.9 ng/mL (week 9 of actual study) | 13.8  (68.9/5.01) | Similar plasma conc. in females at 1.0 mg/kg but no MN response  ANEUGEN |
| 10.0 ng/mL (3+19 hr –S9)  Not determined for 3+19 hr +S9 | 5.01, 10.0, 31.7 ng/mL (3+19 hr –S9)  40% cytotox. at 31.7 ng/mL | 0.4375, 0.875, 1.75, 3.5, 7 mg/kg (iv, 1x daily for 2 days)  Bone marrow sampled ~24 hrs after 2nd injection. | 1.75 mg/ kg | MN (rat bone marrow, males only) | 110 ng/mL (from actual study) |  |
| 10 | BMS-2  (an inhibitor of tyrosine kinase activity) | 4.70 µg/mL (22+0 hr -S9)  Aneugen | 3.91, 4.70, 5.63 µg/mL (22+0 hr –S9)  53% cytotox. at 5.63 µg/mL | CHO-WBL cells | 62.5, 125, 250, and 500 mg/kg oral gavage,1x daily for 3 consecutive days  Bone marrow sampled ~24 hrs after 3rd dose | 250 mg/kg in males  500 mg/kg in females | MN (rat bone marrow)  No other endpoints or tissues studied | 18.7 µg/mL (males);  42.3 µg/mL (females)  Data collected from doses used in study | 3.98  (18.7/4.70) | ANEUGEN |
| 50.2 µg/mL (3+19 hr +S9)  Not determined for 3+19h -S9 | 41.9, 50.2, 60.3 µg/mL (3+19 hr +S9)  60% cytotox. at 60.3 µg/mL |
| 11 | Sodium arsenite | 2 µM (259.8 ng/mL); 48 hr -S9; 20% cytotox.; aneugen & clastogen (approx. 50/50);  (Colognato et al., 2007) | 0.5, 1, 2, 4 µM (48 hr -S9) | HLC | 50 mg/L in drinking water (equivalent to 10 mg/kg/day) for 7 days  (Lewinska et al., 2007) | 10 mg/kg/day.  (only dose tested but 2x control MN frequency) | MN (mouse bone marrow) | 136.5 ng/mL at 5 mg/kg (extrapolated from Cmax at oral dose in mice of 200 µg/kg);  (Twaddle et al., 2018) | 1051  (136.5/0.1299) | CLASTOGEN/ANEUGEN |
| 0.001 µM (129.9 pg/mL); 24 hr -S9; hyperdiploidy; (Ramirez et al., 1997) | 0.001, 0.01, 0.1 µM (24 hr -S9) | HLC | 2.5, 5, 10 mg/kg single dose (sampled 24 & 48 hrs later) or 4 daily doses (sampled 24 hr after last dose);  (Tice et al., 1997) | 5 mg/kg/day in 4-day protocol | MN (mouse bone marrow; decrease in comets (cross-linking) in liver parenchymal cells and bladder |
| 5 µM (649.5 ng/mL), 85% cytotox in 24 hr protocol (aneugen), 35% cytotox in 4 hr protocol (clastogen);  (Yih & Lee, 1999) | 1.25-10 µM for 24 hr or 5-80 µM for 4 hr -S9 | HFW human fibroblasts | 5, 10 mg/kg (single i.p. dose)  (Tinwell et al., 1991) | 5 mg/kg when dissolved in water; negative in corn oil | MN (mouse bone marrow) |
| 12 | Glycidamide | 100 µM (8.7 µg/mL);  24+0 hrs-S9; <2-fold increase  (Le Hegarat et al., 2010) | 100, 250 & 500 µM (24+0 hrs -S9) | HepaRG | 120 or 600 mg/L (25-35 or 88-111 mg/kg/day) drinking water for 28 days, blood sampled 24 hrs later, (Manjanatha et al., 2006) | 88 mg/kg/day | MN (Big Blue mouse retics)  Increased *Hprt* mutations in lymphocytes and *cII* mutations in liver | 587 µM (51.04 µg/mL), extrapolated from Cmax of 0.8 µM at 120 µg/kg oral in mice (Doerge et al., 2005) | 5.87  (51.04/8.7) | Several *in vivo* studies showing MN induction following i.p. or s.c. dosing of mice& rats (Paulsson et al., 2003; Von Tungeln et al., 2009; Husøy et al., 2005), but no plasma conc. data for these routes.  CLASTOGEN according to Dertinger et al. (2019) |
| 250 µM (21.75 µg/mL) for CA; 16+2.5 hrs- S9; clastogen  (Martins et al., 2007) | 1-1000 µM  16+2.5 hrs -S9 | V79 |
| <500 µM (43.5 µg/mL); 4+48 hrs -S9; non-toxic but lowest conc. tested (Koyama et al., 2006) | 500-2500 µM  (4 + 48 hrs -S9) | TK6 |
| 1 mM (87 µg/mL); 24+24 hrs -S9 (Bandarra et al., 2013) | 0.25, 0.5, 1 mM. (24+24 hrs -S9) | MCF10A human mammary epithelial cells | Approx. 5 and 10 mg/kg/day for 2 months via drinking water, blood sampled 24 hrs later. (Mei et al., 2010) | No increase in MN | MN (Big Blue rat retics) Weak increase in *Hprt* mutants in lymphocytes, and *cII* mutations in bone marrow & thyroid |
| Negative up to 1 mM (23+0 hrs -S9) (Baum et al., 2005) | 50, 100, 250, 1000 µM (23+0hrs -S9) | HLC |
| 13 | Hexavalent chromium | 25 nM (0.00735 µg/mL) 48+0 hrs -S9 in TK6 cells;  100 µM (29.4 µg/mL) in HLC (Cemeli et al., 2006) | Potassium dichromate at 75 & 100 µM in HLC; 10 & 25 nM in TK6 cells (48+0 hrs -S9) | HLC, TK6 | Sodium dichromate 62.5-1000 mg/L drinking water over 3 months. (NTP TOX 72) | 62.5 mg/L  (<2-fold increase) in *am3*-C57BL/6 mice  E in B6C3F1 mice; negative in BALB/c mice | MN (mouse blood NCEs) | 0.645 µg/g (~µg/mL) at 62.5 mg/L in drinking water extrapolated from 0.524 µg/g at 30 mg/L and 0.784 µg/g at 100 mg/L  (NTP TOX 72) | 87.8  (0.645/0.00735) | Several *in vivo* studies positive for MN in mice & rats following i.p. dosing (García-Rodríguez et al., 2014; Itoh & Shimada, 1996; Chorvatovicová et al., 1991; Shindo et al., 1989; De Flora et al., 2006), but no plasma conc. data for this route  ANEUGEN |
| 2 µM (0.588 µg/mL), 24 to 26+0 hrs -S9; aneugen (Seoane & Dulout 2001) | Potassium dichromate at 1, 2, 4 µM  (24 to 26+0 hrs -S9) | MRC-5 |
| No sig. induction of MN  (Suzuki et al., 2018) | Sodium dichromate at 2.5, 5, 7.5, 10 µM (4+28 hrs -S9) | Human Nalm-6-MSH cells |
| 14 | Acetaminophen | 15 µg/mL:  24 & 48 hr -S9;  (Matsuoka et al., 1993; Matsushima et al., 1999) | 12.5, 25. 50, 100 µg/mL (48 & 72 hr -S9)  and  15, 30, 60, 120 µg/mL (24 & 48 hr -S9) | CHL | 250, 500, 1000, 2000 mg/kg/day;  daily oral doses for 3 or 28 days (Van der Leede et al., 2020) | 500 mg/kg  (only after 28 days, assoc. with haematotoxicity) | MN (rats, retics.);  Negative for Pig-a mutations, weak but not biologically relevant increases in comets in blood and liver at 1000 mg/kg/day in 3 -day study | 65.75 µg/mL (extrapolated from 1000 mg/kg/day at day 28) | 4.38  (65.75/15) | Multiple publications (see Kirkland et al., 2021) *in vitro* and *in vivo*. Only positive (PROBABLE CLASTOGEN) in p53-competent cells at cytotoxic concentrations due to adverse effects on cellular processes |
| 151.16 µg/mL;  72 hr -S9  (Simkó et. al., 1998) | 45.3-377.6 µg/mL  (24, 48 & 72 hr -S9) | Human amniotic fluid (AFC) cells | 25, 50, 100, 175, 250, 500 mg/kg;  3 oral doses at 4 h intervals; single oral dose of 1500 mg/kg (Thomas, 1995). | 500 mg/kg | MN (rat bone marrow) | 76.8 µg/mL (extrapolated from day 1 data at 1000 mg/kg in Van der Leede et al., 2020) |
| 1511.6 µg/mL;1 hr -S9  (Dunn et al., 1987); | 755.8-3023.2 µg/mL  (1 hr -S9) | NRK-49F rat kidney cells | 5, 50, 100, 150, 200 mg/kg;  single i.p. dose | 100 mg/kg | MN (mouse bone marrow) | Not available for i.p. dosing |
| Negative; 72 hr -S9  (Ibrulj et al., 2007) | 50-200 µg/mL  (72 hr -S9) | HLC |
| Negative; 48 hr -S9  (Müller-Tegethoff et. al., 1995) | 1.5-150 µg/mL  (48 hr -S9) | Primary rat hepatocytes |
| 15 | Ethyl methanesulfonate | Multiple publications but LOEC 1.4 µg/mL for 1 cell cycle -S9 in most detailed study (Doak et al., 2007) | 0.2-2.5 µg/mL (1 cell cycle -S9);  No cytotoxicity | AHH-1 | 50, 100, 200, 400 mg/kg;  Single oral or i.p. dose  (Kondo et al., 1989) | 50 mg/kg | MN (bone marrow, CD-1 and MS/Ae mice) | 34.6 µg/mL extrapolated from 5 mg/kg (Lavé et al., 2009) or 78.12 µg/mL extrapolated from 25 mg/kg/day for 7 or 28 days (Mϋller et al., 2009) | 24.7  (34.6/1.4) | Multiple reports for MN *in vivo*, reviewed by Gocke et al. (2009). Lowest LOEDs included here  CLASTOGEN according to Witt et al. (2008); Dertinger et al. (2019) |
| 50, 100, 200, 300 mg/kg/day;  Once daily oral for 3 days (Witt et al., 2008) | 50 mg/kg/day (rats)  LOED in mice ≥100 mg/kg/day | MN in bone marrow and blood reticulocytes (rats & mice) | No rat plasma data found |
| 16 | Ochratoxin A | 1.5 µM (0.606 µg/mL), 48+0 hrs -S9. (Gonzalez-Arias et al., 2014) | 0.075, 0.15, 1.5, 5, 15 µM (48+0 hrs -S9) | HLC | 3 mg/kg/day oral for 15 days; sampled 12 hrs after last dose. (Abdel-Wahhab et al., 2008) | <3 mg/kg/day (8-fold increase in MN) | MN (rat bone marrow) | <24 µg/mL (extrapolated from 4.15 µg/mL (at 0.5 mg/kg in Vettorazzi et al., 2010) and 4.32 µg/mL (at 0.5 mg/kg in Corcuera et al., 2012) | <39.6  (24/0.606) | Multiple *in vitro* MN studies. Those with lowest LOEC given here.  Also positive *in vivo* MN studies with i.p. dosing of mice but no plasma conc. data for i.p. dosing in mice |
| 5 µg/mL (~2-fold at lowest conc tested), 24+0 hrs -S9. (Ehrlich et al., 2002, confirmed in Knasmuller et al., 2004) | 5, 10, 25, 50 µg/mL (24+0 hrs -S9) | HepG2 | 50, 150, 450 µg/kg/day, oral, 5 days/week for 4 weeks; sampled 1 day later. (Alvarez et al., 2004) | Weak response at 150 µg/kg/day (increase <2-fold in MN-PCE, 2.5-fold in MN-NCE, not sig.); MN lower at 450 µg/kg/day | MN (rat bone marrow) |
| 15 µM (6.06 µg/mL) in both cell lines (Ali et al., 2011) | 5-25 µM  (24+0 hrs -S9, CHO; 27+0 hrs -S9, TK6) | CHO  TK6 | 0.5 mg/kg single oral dose, sampled after 3 & 24 hrs.  (Corcuera et al., 2015) | No increase in MN at 3 or 24 hrs | MN (rat bone marrow) |
| 25 µM (10.1 µg/mL) 48 hrs -S9  (Donmez-Altuntas et al., 2003) | 100 pM-25 µM (48 hrs -S9) | HLC |
| 17 | Benzo(a)pyrene | 0.1 µg/mL (MCL-5, ICR protocol);  0.5 µg/mL (MCL-5); 1.25 µg/mL (AHH-1). (Crofton-Sleigh et al., 1993) | MCL-5 treated 24+24 hrs -S9 & AHH-1 treated 24+18 hrs -S9.  In ICR protocol (MCL-5) concs were 0.1, 0.5, 1.0 µg/mL.  In Swansea protocol (MCL-5 and AHH-1) concs were 0.5, 1.25, 2.5, 5.0, 10.0 µg/mL. | MCL-5  AHH-1 | Mice dosed i.p. or oral with 62.5, 125, 250 & 500 mg/kg. BM sampled 48 hrs later.  (Hayashi et al., 1989) | Clearly +ve in MS/Ae mice but weakly +ve (close to LOED) in CD-1 mice by both routes at 62.5 mg/kg | MN (mouse bone marrow) | <1.146 µg/mL at 62.5 mg/kg (extrapolated from 275 ng/mL after single oral dose of 15 mg/kg)  (Uno et al., 2004) | <11.5  (1.146/0.1) | Many *in vitro* MN studies. Those with lowest LOEC given here.  Many *in vivo* MN studies with i.p. dosing but no plasma conc. data for this route.  CLASTOGEN according to Bryce et al. (2010) |
| 3 µg/mL (3+21 hrs +S9)  (Fowler et al., 2010a) | 3, 6, 9, 12, 24, 33 µg/mL ( 3+21 hrs +S9) | TK6 |
| 20 µM (5.05 µg/mL) in V79 cells +S9 and in V79-hCYP1A2 cells -S9. No MN induced in V79 cells -S9.  (Zhu et al., 2020) | 10. 20 and 40 µM (V79, 3+21 hrs - & + S9); 5, 10 and 20 µM (V79-hCYP1A2, 3+21 hrs -S9). | V79  V79-hCYP1A2 | 62.5, 125 mg/kg/day oral for 28 days. Tested in gpt-delta and non-Tg F344 rats  (Hori et al., 2019) | 125 mg/kg/day. | MN (rat bone marrow).  Also weak +ve at 62.5 mg/kg/day in liver MN. | 12.625 µg/mL at 125 mg/kg (extrapolated from 0.4 pmol/mL, 101 pg/mL, after single oral dose of 4 nmol/kg (1 µg/kg)  (Foth et al., 1988) |
| <25 µM (6.31 µg/mL; 2.5-fold increase at lowest conc.)  (Liu et al., 2019) | 25, 50, 100, 250 µM for 24 hrs. | Human-induced hepatocytes |
| 18 | Cytosine arabinoside | 0.004 µg/mL (24+0 hrs -S9).  (Whitwell et al., 2010b) | 0.001-0.015 µg/mL (24+0 hrs -S9) | V79 | 0.5-400 mg/kg/day  (2 daily i.p. doses). Sampled 6 hrs after second dose.  (Maier & Schmid, 1976) | 1 mg/kg/day | MN (mouse bone marrow) | 0.2 µg/mL at 1 mg/kg (extrapolated from plasma conc. of 20 µg/mL 0.5 hrs after mice dosed i.p. with 100 mg/kg). (Pallavicini & Mazrimas, 1980) | 51.2  (0.2/0.00391) | Several *in vitro* MN studies in HLC, L5178Y, & CHO cells. Lowest LOEC given here.  Other *in vivo* i.p. studies in mice gave strong responses at lowest dose.  CLASTOGEN according to Dertinger et al. (2019) |
| 0.004 µg/mL (24+24 hrs -S9) (Wakata et al., 2006) | 0.00025-4 µg/mL (3+18, 3+21, 3+45, 24+0, 24+18, 24+24 hrs -S9) | CHL |
| 0.00391 µg/mL (TK6, NH32) and 0.00781 µg/mL (CHL) in 24+0 hrs protocol  (Hashimoto et al., 2011) | Cells treated 3+21 hrs or 24+0 hrs -S9 with different ranges of concs. up to 50% cytotoxicity | TK6  p53 null NH32  CHL |
| 19 | Etoposide | 10.2 nM (6 ng/mL); 4+18 hrs -S9  (Boos & Stopper, 2000) | 2, 10.2, 51, 255 nM (4+18 hrs -S9) | L5178Y | 1 mg/kg, single oral dose, sampled 24 hrs later  (Spronck & Kirkland, 2002) | 1 mg/kg  (small but sig increase with niacin-replete, niacin deficient & nicotinic acid supplemented diets) | MN (rat bone marrow) | 46 or 47.3 ng/mL (extrapolated from Cmax of 276 or 284 ng/mL, 30 mins after 6 mg/kg oral dose)  (Li et al., 2007,2009) | 7.67  (46/6) | Many *in vitro* MN studies +ve at lowest conc. tested. Lowest LOEC given here.  Other oral studies in rats gave MN responses at higher doses.  CLASTOGEN according to Dertinger et al. (2019) |
| 12.5 nM (7.4 ng/mL); 24+0 hrs -S9; clastogen (Gollapudi et al., 2019) | 12.5, 25, 50, 100 & 200 nM  (24+0 hrs -S9) | TK6 |
| 20 | Bleomycin/  bleomycin sulfate | 0.0068 µg/mL (24+0 hrs -S9) (Aardema et al., 2006) | 0.0017-500 µg/mL (3+20, 3+21, 3+45, 24+0, 24+20, 24+24 hrs -S9) | CHO | 300 µg/kg bleomycin sulfate, single IV dose.  Sampled 24, 36 & 48 hrs later. (Mozdarani & Saberi, 1994) | 300 µg/kg  (3-fold increase in MN-PCE and MN-NCE) | MN (Syrian albino mice bone marrow) | 0.654-1.194 µg/mL at 300 µg/kg; extrapolated from Cmax 1 min after IV dose of 5 mg/kg was 10.9 µg/mL in C57BL/6 mice and 19.9 µg/mL in BALB/c mice (Groselj et al., 2018) | 96.2  (0.654/0.0068) | Several positive *in vivo* MN studies in mice with i.p. dosing but no plasma conc data by this route.  CLASTOGEN according to Dertinger et al. (2019) |
| * 1. µg/mL   (24+24 hrs -S9) (Oliver et al., 2006) | 0.05-250 µg/mL (3+20, 3+21, 3+45, 24+0, 24+20, 24+24 hrs -S9) | L5178Y |
| 0.125 µg/mL (24+18 hrs -S9) (Wakata et al., 2006) | 0.125-500 µg/mL (3+18, 3+21, 3+45, 24+0, 24+18, 24+24 hrs -S9) | CHL |
| * 1. µg/mL   (3+45 hrs -S9) (Clare et al., 2006) | 0.5-500 µg/mL  (3+26, 3+45, 20+28 hrs -S9) | HLC |
| 0.5 µg/mL estimated from graph (24+0 hrs -S9)  (von der Hude et al., 2000) | 0.01-100 µg/mL bleomycin sulfate (24+0 hrs -S9) | V79 |
| <5 µg/mL for all 3 species of PBLs  (Erexson et al., 1995) | 5-160 µg/mL (4+21 hrs with PHA + undefined time with cytoB) | Human, rat & mouse peripheral blood lymphocytes |
| 21 | Methotrexate | 0.1 µg/mL  (4+20 hrs -S9)  (Le Fevre et al., 2007) | 0.001. 0.1 & 300 µg/mL (4+20 hrs -S9) | TK6 | 0.25-128 mg/kg (single i.p. dose, sampled 30 hrs later), or 0.125-4 mg/kg/day (5 daily i.p. doses, sampled 6 hrs after last dose)  (Yamamoto & Kikuchi, 1981) | 0.25 mg/kg/day in 5-day protocol | MN (mouse bone marrow) | 0.58 µg/mL (extrapolated from 231 µg/mL at single 100 mg/kg i.p. dose (different dosing & feeding schedules)  (Song et al., 1993) | 5.80  (0.58/0.1) | Old *in vitro* CA studies positive only at excessive cytotoxicity.  Many mouse & rat i.p. studies positive at higher doses  CLASTOGEN according to Dertinger et al. (2019) |
| 5 µg/mL (2.2-fold increase; 6+40 hrs +S9)  (Keshava et al., 1998) | 5, 10, 25, 50, 100 µg/mL (6+40 hrs +S9) | V79 |
| 22 | Carbendazim | 2.2 µM (0.42 µg/mL; 44+28 hrs -S9) estimated from graph.  (Frieauff et al., 2013) | 1.0-4.8 µM  (44+28 hrs -S9) | HLC | 50, 100, 500, 1000 mg/kg/day (2 daily oral doses) or 500 mg/kg/day (2 daily i.p. doses), sampled 24 hrs after 2nd dose.  (Seiler, 1976) | 100 mg/kg/day by oral dosing. No MN induced by i.p. dosing. | MN (mouse bone marrow) | 11.5 µg/mL at 100 mg/kg in same study  (Seiler, 1976);  or ~15.0 µg/mL (extrapolated from 500 mg/kg (Jia et al., 2003) | 27.4  (11.5/0.42) | Other *in vitro* MN studies positive at higher concs.  Other rat & mouse *in vivo* MN studies but those with lowest plasma concs. given here  ANEUGEN according to Dertinger et al. (2019) |
| 2.62 µM (0.5 µg/mL; 48+0 hrs -S9). Aneugen. (Elhajouji et al., 1995) | 0.2-10 µM  (48+0 hrs -S9) | HLC | 62.5-2000 mg/kg/day (3 daily oral doses), sampled 3 hrs after 3rd dose. (Ilyushina, 2020) | 125 mg/kg/day | MN (mouse bone marrow) |
| 23 | Taxol | 1 nM (0.854 ng/mL) mouse splenocytes; 5 nM (4.27 ng/mL) HLC (48+0 hrs -S9)  (Steiblen et al., 2005) | 0.5, 1, 2.5, 5, 7.5 nM (48+0 hrs -S9) | HLC  Mouse splenocytes | 10 µg/kg (single i.p. dose); sampled 12, 24 & 36 hrs later. (Jagetia & Nayak, 1996) | 10 µg/kg (2-3-fold increase in MN-PCE at 24 hrs; MN-NCE increased similarly at 24 & 36 hrs) | MN (mouse bone marrow) | 7.2 ng/mL (extrapolated from Cmax of 13.0 µg/mL after single i.p. dose of 18 mg/kg (Innocenti et al., 1995) | 8.43  (7.2/0.854) | Other *in vitro* and *in vivo* studies positive at higher concs. and doses.  ANEUGEN according to Dertinger et al. (2019) |
| 2.5 nM (2.135 ng/mL); 48+0 hrs -S9. Aneugen. (Digue et al., 1999) | 2.5, 5, 7.5, 10 nM (48+0 hrs -S9) | HLC |
| 0.01 µg/mL (4+20 hrs -S9)  (Le Fevre et al., 2007) | 0.001, 0.01, 1.0 µg/mL (4+20 hrs -S9) | TK6 |
| 0.053 µg/mL (24+0 & 24+20 hrs -S9)  (Nesslany & Marzin, 1999) | 0.027-0.427 µg/mL (24+0 or 24+20 hrs -S9) | L5178Y |
| 24 | Colchicine | 3 ng/mL (3+26 hrs -S9)  (Clare et al., 2006) | 0.0015-0.15 µg/mL (3+26, 3+45, 20+28 hrs -S9) | HLC | 1, 2, 4, 8, 16 mg/kg (single oral doses) or 0.25, 0.5, 1, 2 mg/kg (single i.p. doses); sampled 24 hrs later  (Hayashi et al., 1989) | 0.5 mg/kg by i.p. dosing | MN (mouse bone marrow) | 30.5 ng/mL (extrapolated from Cmax of 0.61 µg/mL after single i.p. dose of 10 mg/kg)  (Chen et al., 2007) | 10.2  (30.5/3) | Other *in vitro* and *in vivo* studies positive at higher concs. and doses.  ANEUGEN according to Dertinger et al. (2019) |
| <5 ng/mL (>6-fold increase, 3+24 hrs -S9)  (Fellows & O’Donovan, 2010) | 0.005, 0.0072, 0.01 µg/mL (3+24 hrs -S9) | L5178Y |
| 5 ng/mL (>2-fold increase in MN bis and >4-fold increase in MN monos; 3+27 hrs -S9)  (Elhajouji, 2010) | 0.005-0.5 µg/mL (3+27 hrs -S9) | TK6 |
| 25 | Potassium chromate | 0.0625 µg/mL (<3-fold increase in MN; 24+0 hrs -S9). (Ren et al., 1993) | 0.0625, 0.125, 0.25, 0.5 & 1 µg/mL (24+0 hrs -S9) | Mouse splenocytes | 10, 15, 20, 30, 40, 60 & 80 mg/kg, single i.p. dose. Blood sampled 24, 48, 72 & 96 hrs later. (Awogi et al., 1992) | 10 mg/kg at 48 hr sample. | MN (CD-1 mouse peripheral blood retics) | 68.125 µg/mL (extrapolated from Cr plasma conc and adjusted for chromate at 0.8 mg/kg/day K2CrO4, i.p., for 14 days) (Kargacin et al., 1993) | 1090  (68.125/0.0625) | Other i.p. studies in mice positive for MN at higher doses |
| <20 µM (3.89 µg/mL) for CA excl. gaps (approx. 4-fold increase)  (Douglas et al., 1980) | 20, 40, 60, 80 & 100 µM (6+18 hrs -S9) | HLC |

**B: Data from *in vitro* chromosomal aberration (CAvit) studies**

| **No.** | **Chemical (therapeutic area or proposed use)** | **LOEC for CAvit (µg/mL); treatment conditions; aneugen or clastogen (if determined)** | **Concentrations scored for in CAvit (µg/mL). Other concentrations, treatment times, conditions scored** | ***In vitro* cell type used**  **(e.g. human lymphocytes [HLC], TK6, V79, CHO etc.)** | **Dose levels scored for *in vivo* MN**  **(mg/kg); number of administrations** | **Lowest +ve dose *in vivo* (LOED or lowest dose tested)** | **Endpoint (species, tissue) giving LOED (+ other relevant data)** | **Blood or plasma conc. at LOED (at doses used in study or extrapolated from other doses/studies)** | **Ratio of plasma LOED:LOEC (to 3 significant figures)** | **Comments** |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| 26 | XALKORI  (crizotinib)  Data from Drugs@FDA | 2.5 µg/mL (3+21 hr -S9);  10 µg/mL (3+21 hr +S9);  Negative in 24+0 -S9  But +ve in CHO MNvit at 0.2 µg/mL (aneugen) | * 2.5, 5, 7.5 µg/mL (3+21 hr, –S9) * 1, 5, 10 µg/mL (3+21 hr, +S9) * 1, 1.5, 2.5 µg/mL   (24+0 hr, -S9) | HLC. | 125, 250, 500, and 1000 mg/kg/day (males & females, 2 doses);  25, 100 and 250 mg/kg/day in supplemental study (2 doses) in males | 250 mg/kg/day in males;  (-ve in females up to 1000 mg’kg/day but lower exposure than male) | MN (rat  bone marrow) | 2.25 µg/mL (males) | 11.3  (2.25/0.2) | Kinetochore +ve in MNvit using CHO cells (0.20~0.30 µg/mL)  ANEUGEN |
| 27 | Compound XX  (Anti-inflammatory/  Immunosuppression) | 3 µg/mL (24+0 hr -S9;  2 µg/mL (48+0 hr -S9.  (Structural CA & polyploidy) | * 1,2,3,4, 5   (24+0 and 48+0 hr -S9). | CHL/IU cells | 6.25, 12.5, 25, 50 and 100 (MTD) mg/kg/day (killed 24, 48 and 72 hrs after single oral administration) | 25 mg/kg/day at 24 hrs;  50 mg/kg/day at 48hrs.  Negative at 72hrs. | MN (rat bone marrow) | 17.3-22.2 µg/mL at 25 mg/kg/day | 8.65  (17.3/2) | CLASTOGEN but possible ANEUGEN activity due to polyploidy |
| Negative in short treatments. | * 100, 200, 300, 400, 500 µg/mL (6+21 hr +/- S9) |
| 28 | Agchem development candidate (discontinued) | Structural CA at 12.5 µg/mL; polyploidy at <3.125 µg/mL (4+18 hr -S9) | * 3.125, 6.25, 12.5 µg/mL (4+18 hr -S9) | V79 cells | 1st MN study  50, 100, 200, 600, 1800 mg/kg bw  Single oral 24 hrs | 200 mg/kg bw | MN (mouse bone marrow) | 0.85 µg/mL  Extrapolated from kinetic study in rats performed at 200 mg/kg bw with single oral gavage but using 0.5% CMC with 5% cremophor as a vehicle instead of corn oil as used in the in vivo genotoxicity assays | >17.0  (0.85/0.05) | Fungicidal MoA tubulin polymerase inhibition  ANEUGEN based on tubulin inhibition and polyploidy but also some CLASTOGEN activity |
| Structural CA at  50 µg/mL; polyploidy at <12.5 µg/mL  (4+18 hr +S9) | * 12.5, 25, 50 µg/mL (4+18 hr +S9) | 2nd MN study  50, 100, 200, 400, 800 mg/kg bw  Single oral 24 hrs | 400 mg/kg bw | MN (mouse bone marrow) |
| Polyploidy at <0.05 µg/mL (4+18 hr -S9);  2.5 µg/mL  (4+18 hr +S9) | Additional experiment to determine polyploidy at   * 0.05, 0.25, 0.5, 1, 3, 4, 5 µg/mL (4+18 hr -S9) * 1.25, 2.5, 5, 10, 15, 20 µg/mL (4+18 hr +S9) | CA study  50, 100, 200, 400, 800 mg/kg bw  Single oral 24 hrs | No increase in structural CA at any dose; increased polyploidy <200 mg/kg bw | CA & polyploidy  (mouse bone marrow) |
| 29 | 4-Aminophenol | 5 µg/mL (24+0 hrs -S9)  (Kusakabe et al., 2002) | 2.5, 5, 10 µg/ml (24+0 hrs -S9). Also tested 6+18 hrs - & +S9, & 48+0 hrs -S9 but concs. not given | CHL | 53.6, 107.2, 214.5 mg/kg (once oral). Spleens sampled 2 & 14 days later. (Benning et al., 1994) | 53.6 mg/kg (2-fold increase at day 14) | MN (mouse splenocytes – spleen is primary tumour site) | 22.38 µg/mL (free + bound in whole blood); extrapolated from Cmax of 38 µg/mL after single oral dose to mice of 91 mg/kg. **(**Carraz et al., 1967) | 4.48  (22.38/5) | Also mouse i.p. studies but no plasma conc. data, and rat oral studies giving negative MN results |
| For CA 6 µg/mL (L5178Y), 20 µg/mL (CHO), 4 hrs -S9. Also 6 µg/ml for TK mutations (exceeding GEF) (Majeska & Holden, 1995) | 0.5-14 µg/mL (L5178Y), 10-30 µg/mL (CHO), 4 hrs -S9. CA scored 20 hrs after start of treatment. | CHO  L5178Y | 125, 250, 500 mg/kg (once oral). Sampled 24, 48 & 72 hrs later.  (REACH registration dossier) | <<500 mg/kg (strong +ve, >10-fold)  Bone marrow toxicity at 125 mg/kg at 24 hrs | MN (mouse bone marrow) |
| 5 µg/mL for TK mutations exceeding GEF (4 hrs -S9)  (Oberly et al., 1984) | 2-7 µg/mL (4 hrs -S9). | L5178Y (TK mutations) | 170, 250, 500 mg/kg (once oral). Sampled 24 & 48 hrs later.  (REACH registration dossier) | <<170 mg/kg (strong +ve, 7-fold, at 24 hrs).  Bone marrow toxicity at 500 mg/kg at 48 hrs | MN (mouse bone marrow) |
| 19.2 µg/mL (3 hrs +S9) for TK mutations, but only 5% relative survival (Amacher & Turner, 1982) | 1.9-19.2 µg/mL (3 hrs +S9) | L5178Y |

**C: Data from mouse lymphoma assays (MLA studies)**

| **No.** | **Chemical (therapeutic area or proposed use)** | **LOEC for MLA (µg/mL); treatment conditions; aneugen or clastogen (if determined)** | **Concentrations scored for in MLA (µg/mL). Other concentrations, treatment times, conditions scored** | ***In vitro* cell type used**  **(e.g. human lymphocytes [HLC], TK6, V79, CHO etc)** | **Dose levels scored for *in vivo* MN**  **(mg/kg); number of administrations** | **Lowest +ve dose *in vivo* (LOED or lowest dose tested)** | **Endpoint (species, tissue) giving LOED (+ other relevant data)** | **Blood or plasma conc. at LOED (at doses used in study or extrapolated from other doses/studies)** | **Ratio of plasma LOED:LOEC (to 3 significant figures)** | **Comments** |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| 30 | LRRK2 inhibitor for Parkinson disease | 3 µg/mL (24 hr -S9 in MLA)  Increases predominantly small colonies | * 1.0, 2.0, 3.0, 4.0 µg/mL (limited by toxicity) (24 hr -S9) * 15, 30, 60, 90, 120 µg/mL (limited by toxicity) (3hr –S9) * 15, 30, 60, 90, 120, 140 µg/mL (limited by toxicity) (3hr +S9) | L5178Y cells | 125, 250, and 500 (MTD) mg/kg/day (3 admins at 0, 24 and 44 hrs) | 125 mg/kg/day | MN (Rat bone marrow). Positive in liver comet | 18.7 µg/mL (linear extrapolation from mean plasma concs at 500 mg/kg/day) | 6.23  (18.7/3) | Equivocal for MN (all doses); statistically significant increases in comets at 250 and 500 mg/kg/day |
| 31 | Compound G  (Oncology) | 0.6 µg/mL  (24 hr -S9)  Also +ve with 3 hr treatments | * 0.1, 0.5, 1, 2, 3, 4, 5, 6, 7 µg/mL (3 hr -S9) limited by cytotoxicity * 1, 2.5, 5, 6, 7, 8, 9, 10 µg/mL (3 hr +S9) limited by cytotoxicity * 0.025, 0.05, 0.1, 0.2, 0.4, 0.6, 0.8, 1 µg/mL (24 hr -S9) limited by cytotoxicity | L5178Y tk +/- 3.7.2C | 40, 80, 160, 320 mg/kg (1 admin; harvest 48 hr post-dose; males) | 80 mg/kg | MN (mouse bone marrow) | Group mean Cmax 2.467 µg/mL  Data collected from doses used in study | 4.11  (2.467/0.6) |  |