Thienpont et al.: Novel Prediction Models for Genotoxicity Based on Biomarker Genes in Human HepaRG™ Cells

Supplementary Data

Graphical abstract

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Tab. S1: List of 19 genotoxic reference chemicals with main mechanism of action

| Chemical name | In vitro genotoxicity | In vivo genotoxicity | Main mechanism of action | CAS number | References |
|---------------|-----------------------|----------------------|--------------------------|------------|------------|
| Allatoin B (AFB) (pro-gtx) | + | + | Bulky adduct formation | 1162-65-8 | Vinken et al., 2008; Kirkland et al., 2016 |
| Benzoaprene (BAP) (pro-gtx) | + | + | Polycyclic aromatic hydrocarbon, bulky adduct formation | 50-32-8 | Vinken et al., 2008; Kirkland et al., 2016 |
| Bleomycin sulfate (BLE) | + | + | Radical generator causing DNA strand breaks (radiomimetic) | 9041-93-4 | Miller, M. G. et al., 1986; Mzodzani and Saberi, 1994; Kirkland et al., 2008; Miller, K., 1991 |
| Cadmium chloride (CAD) | - | + | DNA repair inactivator, cell cycle inducer, p53 inhibitor | 10108-64-2 | Kirkland et al., 2016 |
| Cisplatin (CIS) | + | + | Cross-linking agent | 15663-27-1 | Kirkland et al., 2016 |
| Cyclophosphamide (CPM) (pro-gtx) | + | + | Alkylating agent | 50-18-0 | Vinken et al., 2008; Kirkland et al., 2016 |
| Dimethylnitrosamine (DMN) (pro-gtx) | + | + | Alkylating agent | 62-75-9 | Vinken et al., 2008; Kirkland et al., 2016 |
| Methyl methanesulfonate (MMS) | + | + | N7 alkylation, replication fork impairment | 66-27-3 | Kirkland et al., 2016 |
| 2-Nitrofluorene (2-NF) | + | + | Bulky adduct formation | 607-57-8 | Vinken et al., 2008 |
| 4-Methylthioamino-1-3-pyridyl-1-butanolone (NNK) (pro-gtx) | + | + | Bulky adduct formation | 64091-91-4 | Vinken et al., 2008 |
| Vinblastine sulfate (VIN) | - | + | tubulin polymerization inhibitor | 143-67-9 | Kirkland et al., 2016 |
| Zidovudine (ZID) | + | + | Antimetabolite, nucleoside analogue | 30516-87-1 | Kirkland et al., 2016 |
| Glycidol (GLY) | + | + | Clastogen that binds to DNA | 556-52-5 | Mertens et al, 2017; IARC, 2000 |
| 4-Aminophenol (4AP) | - | + | Alkylation agent | 123-30-8 | Mertens et al, 2017; EFSA, 2007; SCCP, 2005; SCCS, 2011b |
| 2,4-Diaminodtoluene (DAT) (pro-gtx) | + | + | DNA adduct formation | 95-80-7 | Kirkland et al., 2016 |
| Ethyl methanesulfonate (EMS) | + | + | Alkylation agent | 62-50-0 | Miller, 1991; Kamber, et al. 2009 |
| 1-Ethyl-1-nitrosourea (ENU) | + | + | Alkylation agent | 759-73-9 | Kirkland et al., 2016 |
| Etosiposide (ETO) | + | + | Topoisomerase II inhibitor | 33419-42-0 | Kirkland et al., 2016 |
| Chloramphenicol (CAM) | - | + | Clastogen that binds to DNA | 56-75-7 | Kirkland et al., 2008 |
Tab. S2: List of 19 non-genotoxic reference chemicals with applicability domain

| Chemical name                | In vitro genotoxicity | In vivo genotoxicity | Main mechanism of action                  | CAS number   | References                                      |
|------------------------------|-----------------------|----------------------|-------------------------------------------|--------------|------------------------------------------------|
| Ampicillin trihydrate (AMP)  | -                     | -                    | Drug/beta-lactam antibiotic               | 7177-48-2    | Kirkland et al., 2016                           |
| Caprolactam (CAP)            | -                     | -                    | Synthetic polymer                         | 105-60-2     | Greene et al., 1979; Hastwell et al., 2006     |
| Clonidine (CND)              | -                     | -                    | Drug/α2 adrenergic agonist                | 4205-90-7    | Vinken et al., 2008                             |
| D-Mannitol (DMO)             | -                     | -                    | Sweetener                                 | 69-65-8      | Vinken et al., 2008; Kirkland et al., 2016     |
| Hydroxybenzomorpholine (HBM) | +                     | -                    | Oxidative hair dye                        | 26021-57-8   | SCCP, 2006                                     |
| 1-naphthol (NAP)             | -                     | +                    | Oxidative hair dye/ insecticide           | 90-15-3      | SCCP, 2008                                     |
| Sodium chloride (NCL)        | -                     | -                    | Condiment/food preservative               | 7647-14-5    | Hastwell et al., 2006                          |
| Nifedipine (NFE)             | -                     | -                    | Drug/antihypertensive                     | 21829-25-4   | Vinken et al., 2008                            |
| Sodium diclofenac (SDF)      | -                     | -                    | Non-steroidal anti-inflammatory drug      | 15307-79-6   | Vinken et al., 2008                            |
| Sorbitol (SOR)               | -                     | -                    | Sweetener/sugar alcohol                   | 50-70-4      | Pottenger et al., 2007                         |
| Tolbutamide (TBT)            | -                     | -                    | Drug/hypoglycemic potassium channel blocker | 64-77-7      | Vinken et al., 2008                            |
| Triclosan (TRI)              | -                     | +                    | Cosmetic preservative                     | 3380-34-5    | SCCS, 2011a                                    |
| 4-Methyl-2-pentanol (4M2P)   | -                     | NA                   | Food contact material                     | 108-11-2     | EFSA, 2017                                     |
| Melamine (MELA)              | -                     | -                    | Additive in resins, plasticizers, paint   | 108-78-1     | Kirkland et al., 2016                          |
| 2-Methyl-1-propanol (2M1P)   | -                     | -                    | Food contact material                     | 78-83-1      | Api et al., 2019; ECHA, 2011                   |
| Phthalimide (PHTH)           | -                     | NA                   | Food contact material                     | 85-41-6      | ECHA, 2007                                     |
| 4-Chlororesorcinol (4CR)     | -                     | +                    | Oxidative hair dye component              | 95-88-5      | SCCS, 2010b                                    |
| Anthranilic acid (ANT)       | -                     | +                    | Intermediate in azo dyes and saccharin production | 118-92-3     | Kirkland et al., 2016                          |
| Basic orange 31 (BOR)        | +                     | -                    | (Oxidative) hair dye component            | 97404-02-9   | SCCS, 2010a                                    |
Tab. S3: List of down- and upregulated GENOMARK biomarker genes and corresponding ensembl gene IDs according to HC analysis after exposure to a genotoxicant

| Downregulated genes | Ensembl gene ID | Upregulated genes | Ensembl gene ID |
|---------------------|-----------------|-------------------|-----------------|
| GENOMARK gene       |                 | GENOMARK gene     |                 |
| MUC15               | ENSG00000169550 | ABCA12            | ENSG00000144452 |
| GPD1                | ENSG00000167588 | ADORA2B           | ENSG00000170425 |
| TNFRSF11B           | ENSG00000164761 | AKR1B1            | ENSG00000085662 |
| ENSG0261578         | ENSG00000261578 | AREG              | ENSG00000109321 |
| FOLH1               | ENSG00000086205 | ARL14             | ENSG00000179674 |
| RARRES1             | ENSG00000118849 | CCDC178           | ENSG00000169660 |
| HLF                 | ENSG00000108924 | CDIP1             | ENSG00000089486 |
| PPP1R1A             | ENSG00000135447 | CEL               | ENSG00000170835 |
| PALMD               | ENSG00000099260 | CLCA2             | ENSG00000137975 |
| MRC1                | ENSG00000260314 | DGKA              | ENSG00000065357 |
| PPP1R3B             | ENSG00000173281 | DRAxin            | ENSG00000162490 |
| PIK3C2G             | ENSG00000139144 | DSE               | ENSG00000111817 |
| C19orf80            | ENSG00000130173 | DUSP14            | ENSG00000276023 |
| EXT1                | ENSG00000182197 | E2F7              | ENSG00000165891 |
| SPP2                | ENSG00000072080 | ENSG0260912       | ENSG00000260912 |
| CYP39A1             | ENSG00000146233 | FDXR              | ENSG00000161513 |
| ADH4                | ENSG00000198099 | FOSL1             | ENSG00000175592 |
| C8B                 | ENSG00000021852 | G6PC              | ENSG00000131482 |
| FREM2               | ENSG00000150893 | GDF15             | ENSG00000130513 |
| GPAM                | ENSG00000119927 | GLIPR2            | ENSG00000122694 |
| C10orf11            | ENSG00000148655 | GPR87             | ENSG00000138271 |
| NCKAP5              | ENSG00000176771 | HERC5             | ENSG00000138646 |
| THRSP               | ENSG00000151365 | IL12A             | ENSG00000168811 |
| AQP9                | ENSG00000103569 | KRT17             | ENSG00000128422 |
| HSD11B1             | ENSG00000117594 | LAMP3             | ENSG00000078081 |
| GOS2                | ENSG00000123689 | LINC01503         | ENSG00000233901 |
| PAGE4               | ENSG00000101951 | MFSD2A            | ENSG00000168389 |
| ONEcut1             | ENSG00000169856 | MICALL1           | ENSG00000100139 |
| SLC22A7             | ENSG00000137204 | NBEAP1            | ENSG00000258590 |
| SLC39A11            | ENSG00000133195 | PMAIP1            | ENSG00000141682 |
| ADH1C               | ENSG00000248144 | PPP1R14C          | ENSG00000198729 |
| CFHR3               | ENSG00000116785 | PROX1             | ENSG00000117707 |
| SLC25A33            | ENSG00000171612 | PVRL4             | ENSG00000143217 |
| ENSG0261051         | ENSG00000261051 | RRAD              | ENSG00000166592 |
| TTPA                | ENSG00000137561 | S100A2            | ENSG00000196754 |
| HFE2                | ENSG00000168509 | SFN               | ENSG00000175793 |
| CYP2A6              | ENSG00000255974 | SPAG1             | ENSG00000104450 |
| INHBE               | ENSG00000139269 | SYNC               | ENSG00000162520 |
| CYP8B1              | ENSG00000180432 | SYTL1             | ENSG00000142765 |
| ENSG0259347         | ENSG00000259347 | TMEM185B          | ENSG00000226479 |
| LIPC                | ENSG00000116603 | TREM2             | ENSG00000095970 |
| SLC22A1             | ENSG00000175003 | VSIG1             | ENSG00000101842 |
Fig. S1: Pearson’s correlation analysis on the individual predictions for the 10 “misleading positives” using support vector machine (SVM) and random forest (RF)
The dots represent the predictions made by SVM and RF, HBM, 2M4I, 4A3N, SoB, DHA, tBHQ, GLU, SoS and EUG (n = 3) and for 1-NAP (n = 4).

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