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## Supplementary data to:

## Original article:

# MIXTURE EFFECTS OF CO-FORMULANTS AND TWO PLANT PROTECTION PRODUCTS IN A LIVER CELL LINE

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**Raw data tables concerning Figure 1:** Cytotoxic effects of Adexar® (red); its active substances' mix (epoxiconazole and fluxapyroxad (green)); and a co-formulant of Adexar® (blue) on HepaRG cells after 24 h treatment obtained from NRU assay. Concentration data were transformed using X=log(X) and then normalized to the solvent control. Error bars indicate standard deviation, n=2 biological replicates each performed with 6 technical replicates. (Concentration of products expressed as the active substances' concentration within the product). Adexar® (a) contains the active substances fluxapyroxad and epoxiconazole in equimolar proportions (each 62.5 g/L).

**Table 1:** *Flourescent readings from Neutral red assay of HepaRG cells after 24 h treatment with Adexar® (1), epoxiconazole and fluxapyroxad in combination (2) and a co-formulant of Adexar®, co-formulant A (3) in concentration ranges from 0.5 to 62.5, 1.25 to 125 and 2 to 250 mg/L respectively.*

| Adexar® | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Concentration mg/L | | | | | | | | | | | |
| | 62,5 | 31,25 | 12,5 | 6,25 | 2,5 | 1,25 | 0,5 | Positive control | Solvent Control | Media control | Blank |
| Sample 1 | 1180 | 650 | 2083 | 3960 | 5420 | 5587 | 5801 | 354 | 5563 | 5729 | 263 |
| | 1138 | 424 | 2568 | 4467 | 5395 | 5112 | 5559 | 489 | 5609 | 5474 | 209 |
| | 799 | 867 | 1898 | 3882 | 5240 | 5407 | 5406 | 295 | 5326 | 5367 | 199 |
| | 565 | 650 | 1772 | 4140 | 5347 | 5537 | 5690 | 505 | 5559 | 5644 | 353 |
| | 525 | 111 | 1432 | 4052 | 5520 | 5369 | 5459 | 115 | 5640 | 5667 | 85 |
| | 628 | 168 | 2061 | 4329 | 5347 | 5443 | 5482 | 289 | 5543 | 5563 | 105 |
| Sample 2 | 548 | 193 | 830 | 4022 | 5713 | 5805 | 5762 | 145 | 5772 | 5582 | 110 |
| | 773 | 506 | 1972 | 5121 | 5585 | 5840 | 5818 | 219 | 5838 | 5743 | 203 |
| | 1146 | 777 | 1828 | 5297 | 5777 | 5779 | 5819 | 249 | 5810 | 5731 | 108 |
| | 1277 | 641 | 1485 | 4576 | 5737 | 5751 | 5727 | 303 | 5717 | 5871 | 247 |
| | 948 | 484 | 2560 | 5442 | 5914 | 5917 | 5794 | 273 | 5784 | 5776 | 89 |
| | 720 | 222 | 1015 | 5098 | 5698 | 5821 | 5731 | 300 | 5659 | 5645 | 805 |

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| Epoxiconazole and fluxapyroxad | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Concentration mg/L | | | | | | | | | | | |
| | 125 | 62.5 | 31.25 | 12.5 | 6.25 | 2.5 | 1.25 | Positive control | Solvent control | Media Control | Blank |
| Sample 1 | 307 | 1335 | 5047 | 5413 | 5418 | 5433 | 5271 | 110 | 5514 | 5501 | 84 |
| | 956 | 1734 | 4189 | 5019 | 5463 | 5457 | 5408 | 258 | 5670 | 5644 | 257 |
| | 619 | 1804 | 5043 | 5283 | 5368 | 5404 | 5357 | 136 | 5561 | 5692 | 323 |
| | 642 | 1839 | 4876 | 5302 | 5487 | 5321 | 5370 | 145 | 5626 | 5578 | 191 |
| | 807 | 2042 | 5023 | 5321 | 5189 | 5468 | 5596 | 191 | 5705 | 5656 | 97 |
| | 877 | 1201 | 4727 | 5186 | 5310 | 5299 | 5337 | 159 | 5418 | 5294 | 77 |
| Sample 2 | 321 | 748 | 5365 | 5740 | 5752 | 5786 | 5807 | 170 | 5670 | 5678 | 99 |
| | 386 | 587 | 5440 | 5636 | 5626 | 5715 | 5651 | 255 | 5723 | 5732 | 129 |
| | 363 | 479 | 5568 | 5664 | 5748 | 5737 | 5895 | 229 | 5928 | 5799 | 82 |
| | 574 | 783 | 5573 | 5753 | 5633 | 5826 | 5788 | 202 | 6004 | 5868 | 90 |
| | 302 | 785 | 5344 | 5768 | 5837 | 5894 | 5905 | 164 | 6094 | 6058 | 319 |
| | 385 | 460 | 5090 | 5473 | 5672 | 5662 | 5660 | 314 | 5818 | 5757 | 145 |

| Co-formulant A | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Concentration mg/L | 250 | 125 | 50 | 25 | 10 | 5 | 2 | Positive control | Solvent control | Media control | Blank |
| Sample 1 | 178 | 206 | 5756 | 5728 | 5691 | 5637 | 5702 | 120 | 5589 | 5714 | 92 |
| | 308 | 157 | 5855 | 5737 | 5715 | 5717 | 5735 | 299 | 5716 | 5757 | 111 |
| | 182 | 154 | 5864 | 5743 | 5752 | 5664 | 5666 | 135 | 5786 | 5794 | 129 |
| | 186 | 176 | 5796 | 5768 | 5730 | 5719 | 5608 | 408 | 5798 | 5858 | 110 |
| | 166 | 147 | 5808 | 5768 | 5833 | 5773 | 5806 | 174 | 5770 | 5766 | 115 |
| | 167 | 149 | 5723 | 5659 | 5710 | 5719 | 5731 | 187 | 5719 | 5673 | 104 |
| Sample 2 | 519 | 429 | 5653 | 5687 | 5674 | 5714 | 5652 | 426 | 5747 | 5636 | 155 |
| | 659 | 488 | 5481 | 5559 | 5618 | 5666 | 5686 | 618 | 5764 | 5697 | 143 |
| | 755 | 785 | 5556 | 5588 | 5601 | 5441 | 5630 | 624 | 5699 | 5739 | 183 |
| | 658 | 690 | 5464 | 5628 | 5681 | 5511 | 5595 | 556 | 5540 | 5661 | 551 |
| | 574 | 546 | 5368 | 5489 | 5380 | 5461 | 5422 | 425 | 5378 | 5202 | 257 |
| | 313 | 261 | 5468 | 5448 | 5589 | 5643 | 5695 | 260 | 5670 | 5586 | 160 |

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**Raw data tables concerning Figure 1:** Cytotoxic effects of Priori Xtra®(red); its active substances‘ mix (cyproconazole and azoxystrobin, green) and a co-formulant (blue) of Priori Xtra® on HepaRG cells after 24 h treatment obtained from NRU assay. Concentration data were transformed using X=log(X) and then normalized to the solvent control. Error bars indicate standard deviation, n=2 biological replicates each performed with 6 technical replicates. (Concentration of Priori Xtra® expressed as the active substances' concentration within the product). Priori Xtra® (b) contains ready-to-use 80 g/L cyproconazole and 200 g/L azoxystrobin.

Only the concentration of cyproconazole is shown in case of both the mixture and the product.

**Table 2:** *Flourescent readings from Neutral red assay of HepaRG cells after 24 h treatment with Priori Xtra® (1), cyproconazole and azoxystrobin in combination (2) and a co-formulant of Priori Xtra®, co-formulant 1 (3) in concentration ranges from 0.32 to 40, 0.32 to 40 and 2 to 250 mg/L respectively.*

| Priori Xtra® | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Concentration mg/L | | | | | | | | | | | |
| | 40 | 16 | 8 | 3.2 | 1.6 | 0.64 | 0.32 | Positive control | Solvent control | Media control | Blank |
| Sample 1 | 223 | 2017 | 4506 | 5995 | 6159 | 6128 | 6128 | 247 | 6045 | 5992 | 148 |
| | 172 | 1846 | 4260 | 5971 | 6055 | 6139 | 6149 | 230 | 6145 | 6086 | 177 |
| | 217 | 2214 | 4538 | 5807 | 5992 | 5955 | 5733 | 215 | 5800 | 5916 | 126 |
| | 536 | 2667 | 4405 | 5924 | 6068 | 6093 | 5980 | 343 | 6101 | 6123 | 134 |
| | 227 | 1552 | 4612 | 5969 | 6107 | 6041 | 5972 | 206 | 6092 | 6055 | 152 |
| | 148 | 2047 | 4045 | 5823 | 5878 | 5789 | 5827 | 294 | 5834 | 5807 | 209 |
| Sample 2 | 3384 | 2608 | 2255 | 4728 | 5866 | 5325 | 5411 | 2250 | 5499 | 5328 | 1557 |
| | 915 | 1040 | 2738 | 5749 | 5890 | 5829 | 5802 | 2604 | 5478 | 5445 | 3089 |
| | 1261 | 2619 | 2086 | 4206 | 3795 | 5113 | 5529 | 4713 | 5538 | 5833 | 240 |
| | 2819 | 1357 | 4013 | 3722 | 4276 | 5635 | 5793 | 3480 | 5239 | 5581 | 1843 |
| | 3453 | 3622 | 1883 | 5210 | 5500 | 5038 | 5271 | 2367 | 5288 | 5224 | 2729 |
| | 748 | 454 | 1093 | 4742 | 4620 | 5630 | 5536 | 1097 | 5220 | 5449 | 1435 |

| Azoxystrobin and cyproconazole | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Concentration mg/L | | | | | | | | | | | |
| | 40 | 16 | 8 | 3.2 | 1.6 | 0.64 | 0.32 | Positive control | Solvent control | Media control | Blank |
| Sample 1 | 4632 | 5561 | 5317 | 5272 | 5490 | 5606 | 5235 | 935 | 5718 | 5486 | 1246 |
| | 4588 | 5373 | 5365 | 5080 | 5308 | 5077 | 5252 | 2008 | 5367 | 5700 | 199 |
| | 4256 | 5828 | 5741 | 5382 | 5104 | 5275 | 5244 | 1405 | 5295 | 5018 | 2042 |
| | 4075 | 6807 | 5077 | 5530 | 5228 | 5454 | 5033 | 3217 | 5122 | 5360 | 1115 |
| | 4220 | 5122 | 5458 | 5603 | 5096 | 4960 | 5077 | 290 | 5028 | 5206 | 628 |
| | 4231 | 4433 | 4999 | 4934 | 4915 | 4965 | 5057 | 847 | 5072 | 5019 | 1408 |
| Sample 2 | 4783 | 5718 | 5934 | 5892 | 5908 | 5876 | 5810 | 200 | 5796 | 5823 | 140 |
| | 4834 | 5573 | 5933 | 5877 | 5831 | 5822 | 5891 | 226 | 5815 | 5892 | 116 |
| | 4854 | 5653 | 5836 | 5848 | 5800 | 5840 | 5854 | 162 | 5757 | 5760 | 834 |
| | 4955 | 5690 | 5942 | 5867 | 5937 | 5658 | 5813 | 254 | 5930 | 5799 | 145 |
| | 5028 | 5635 | 5947 | 5853 | 5843 | 5786 | 5906 | 161 | 5941 | 5892 | 220 |
| | 4536 | 5345 | 5591 | 5651 | 5612 | 5628 | 5556 | 546 | 5653 | 5701 | 154 |

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| Co-formulant 1 | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Concentration mg/L | 63.25 | 25.30 | 12.65 | 5.06 | 2.53 | 1.01 | 0.51 | Positive control | Solvent control | Media control | Blank |
| Sample 1 | 1186 | 4716 | 5057 | 5202 | 5188 | 5522 | 5052 | 2067 | 1059 | 5307 | 2336 |
| | 4503 | 4900 | 5471 | 5038 | 5244 | 5039 | 5306 | 1501 | 5128 | 5618 | 2111 |
| | 5215 | 5161 | 5510 | 5046 | 5446 | 5301 | 5383 | 1470 | 5281 | 5116 | 1611 |
| | 3314 | 4745 | 5143 | 5497 | 4694 | 5258 | 5242 | 3407 | 5317 | 5386 | 762 |
| | 2353 | 5319 | 5263 | 5342 | 5147 | 5408 | 5679 | 1861 | 5341 | 5315 | 890 |
| | 1401 | 4321 | 5107 | 5429 | 5207 | 6150 | 5476 | 3862 | 5544 | 4708 | 1270 |
| Sample 2 | 1611 | 5395 | 5651 | 5801 | 5785 | 5800 | 5654 | 177 | 5812 | 5611 | 191 |
| | 2026 | 5396 | 5631 | 5686 | 5618 | 5573 | 5612 | 116 | 5514 | 5639 | 81 |
| | 2061 | 5475 | 5520 | 5565 | 5683 | 5651 | 5494 | 106 | 5587 | 5500 | 252 |
| | 1709 | 5444 | 5631 | 5699 | 5585 | 5604 | 5545 | 146 | 5706 | 5682 | 96 |
| | 1225 | 5303 | 5247 | 5628 | 5340 | 5490 | 5622 | 123 | 5607 | 5642 | 107 |
| | 1208 | 5475 | 5586 | 5609 | 5630 | 5591 | 5631 | 114 | 5606 | 5635 | 71 |

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**Raw data tables concerning Figure 5:** Treatment-related changes in protein concentration in HepaRG cells. Results were generated based on an MS-based immunoassay. Cytochrome P450 enzymes and the respective ABC transporter were quantified after 24 h treatment with the substances Adexar® at 0.625 mg/L and 1.25 mg/L (expressed as the active substances' concentration within the product); and co-formulant A of Adexar® (5.0 mg/L).
Data shown are the mean +SD (3 technical replicates) for all enzymes quantified. The solvent control (0.4 % DMSO) is indicated as control.

**Table 3:** *Protein concentration of cytochrome P450 enzymes and ABC transporters generated using an MS-based immunoassay in cells treated with Adexar® at 0.625 mg/L and 1.25 mg/L (expressed as the active substances' concentration within the product); and co-formulant A of Adexar® (5.0 mg/L). LLOQ= lower limit of quantification; ULOQ= upper limit of quantification; LOQ= limit of quantification. Replicates indicated as a, b and c.*

| [fmol/µg] | LLOQ [fmol/µg] | ULOQ [fmol/µg] | Control-a | Control-b | Control-c | Adexar-0.625-a | Adexar-0.625-b | Adexar-0.625-c | Adexar-1.25- a | Adexar-1.25-b | Adexar-1.25-c | co-for-mulant A a | co-for-mulant A b | co-for-mulant A c |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Total amount of protein | NA | NA | 0.58 | 0.575 | 0.675 | 0.6 | 0.583 | 0.678 | 0.689 | 0.687 | 0.527 | 0.561 | 0.518 | 0.603 |
| CYP1A1 | 0.0075 | 50 | < LOQ | < LOQ | < LOQ | 0.01 | 0.01 | 0.01 | 0.02 | 0.02 | 0.02 | < LOQ | < LOQ | < LOQ |
| CYP1A2 | 0.0075 | 50 | 0.01 | 0.01 | < LOQ | 0.03 | 0.04 | 0.03 | 0.04 | 0.03 | 0.02 | < LOQ | < LOQ | < LOQ |
| CYP2B6 | 0.206 | 50 | < LOQ | < LOQ | < LOQ | 0.53 | 0.61 | 0.52 | 0.45 | 0.45 | 0.43 | 0.82 | 0.62 | 0.58 |
| CYP2C8 | 0.023 | 50 | 1 | 1.35 | 1.03 | 1.72 | 1.68 | 1.52 | 1.39 | 1.39 | 1.04 | 2.29 | 1.86 | 1.75 |
| CYP2C9 | 0.206 | 50 | 5.7 | 6.45 | 4.41 | 5.1 | 5.5 | 5.05 | 4.3 | 3.85 | 4.11 | 6.72 | 5.8 | 4.75 |
| CYP2C18 | 0.0685 | 50 | 0.08 | 0.09 | 0.08 | < LOQ | < LOQ | < LOQ | < LOQ | < LOQ | < LOQ | < LOQ | < LOQ | < LOQ |
| CYP2C19 | 0.023 | 50 | 0.83 | 0.56 | 0.62 | 0.42 | 0.38 | 0.42 | 0.3 | 0.4 | 0.46 | 1.24 | 0.75 | 0.9 |
| CYP2D6 | 0.206 | 50 | < LOQ | < LOQ | < LOQ | < LOQ | < LOQ | < LOQ | < LOQ | < LOQ | < LOQ | < LOQ | < LOQ | < LOQ |
| CYP2E1 | 0.023 | 50 | 0.64 | 0.95 | 0.57 | 0.75 | 0.89 | 0.81 | 0.63 | 0.67 | 0.48 | 0.99 | 0.86 | 0.65 |
| CYP2F1 | 0.206 | 50 | < LOQ | < LOQ | < LOQ | < LOQ | < LOQ | < LOQ | < LOQ | < LOQ | < LOQ | < LOQ | < LOQ | < LOQ |
| CYP3A4 | 0.0025 | 50 | 2.35 | 3.57 | 2.25 | 11.65 | 12.65 | 9.45 | 11.65 | 11.45 | 10.25 | 6.54 | 5.75 | 4.3 |
| CYP3A5 LPNK | 0.206 | 50 | < LOQ | < LOQ | < LOQ | < LOQ | < LOQ | < LOQ | < LOQ | < LOQ | < LOQ | <LOQ | <LOQ | < LOQ |
| CYP3A7 | 0.206 | 50 | <LOQ | < LOQ | < LOQ | < LOQ | < LOQ | < LOQ | < LOQ | < LOQ | < LOQ | < LOQ | < LOQ | < LOQ |
| CYP3A43 | 0.206 | 50 | < LOQ | < LOQ | < LOQ | < LOQ | < LOQ | < LOQ | < LOQ | < LOQ | < LOQ | < LOQ | < LOQ | < LOQ |
| ABCB1 | 0.0685 | 50 | 1.11 | 1.29 | 1.06 | 1.57 | 1.64 | 1.69 | 1.9 | 1.61 | 1.6 | 2.05 | 1.57 | 1.44 |
| ABCC2 | 0.2600 | 21 | 1.68 | 1.87 | 1.8 | 2.2 | 2.77 | 2.49 | 2.3 | 2.41 | 2.75 | 2.91 | 2.19 | 2.18 |
| ABCC3 | 0.2775 | 67 | 1.12 | 1.02 | 0.79 | 0.93 | 1.06 | 1.29 | 0.9 | 1.09 | 0.89 | 1.29 | 0.96 | 1.13 |

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**Raw data tables concerning Figure 6:** Active substances detected in HepaRG cells after 24 h treatment with Adexar® and its active substances' mix (epoxiconazole and fluxapyroxad), and Priori Xtra® and its active substances' mix (azoxystrobin and cyproconazole) using an LC-MS/MS system. n=3 biological replicates and each sample was injected twice.

**Table 4:** *Concentration (ng/ml) of active substances detected in cells, medium and pbs (used for washing cells) after 24 h treatment with Adexar®, its active substances' mix (epoxiconazole and fluxapyroxad), and Priori Xtra® and its active substances' mix (azoxystrobin and cyproconazole). Epoxy= epoxiconazole, fluxa= fluxapyroxad, azoxy= azoxystrobin, cypro 1= cproconazole 1 and cypro 2= cyproconazole 2.*

| | | cells | | | cells injection 2 | | | medium | | | medium injection 2 | | | pbs | | | pbs injection 2 | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| mix | epoxi | 721 | 592 | 614 | 718 | 589 | 598 | 728 | 679 | 695 | 727 | 672 | 691 | 127 | 202 | 214 | 132 | 210 | 227 |
| | fluxa | 341 | 281 | 295 | 298 | 245 | 249 | 427 | 341 | 338 | 387 | 300 | 298 | 79,1 | 113 | 116 | 67,8 | 97,2 | 102 |
| product (Adexar®) | epoxi | 884 | 1030 | 1108 | 873 | 1004 | 1087 | 1552 | 1622 | 1696 | 1551 | 1605 | 1693 | 666 | 657 | 561 | 698 | 666 | 590 |
| | fluxa | 718 | 784 | 855 | 647 | 711 | 772 | 1205 | 1310 | 1297 | 1126 | 1198 | 1204 | 578 | 599 | 548 | 524 | 528 | 508 |
| mix | Azoxy | 8,38 | 10,1 | 21,5 | 6,22 | 7,56 | 16,8 | 8,26 | 7,93 | 12,9 | 6,48 | 6,4 | 10,4 | 2,34 | 1,64 | 6,3 | 1,64 | 1,12 | 4,65 |
| | cypro 1 | 216 | 212 | 214 | 192 | 185 | 196 | 890 | 908 | 908 | 813 | 822 | 831 | 259 | 239 | 298 | 234 | 215 | 271 |
| | cypro2 | 219 | 213 | 238 | 188 | 183 | 204 | 601 | 613 | 634 | 525 | 536 | 561 | | | | 158 | 146 | 196 |
| product (Priori® Xtra) | Azoxy | 107 | 102 | 219 | 0 | 79,1 | 178 | 413 | 469 | 446 | 361 | 414 | 390 | 107 | 131 | 116 | 84,6 | 106 | 91,5 |
| | cypro 1 | 339 | 299 | 376 | 0 | 271 | 344 | 1769 | 1786 | 1769 | 1630 | 1660 | 1631 | 467 | 509 | 466 | 420 | 451 | 416 |
| | cypro2 | 547 | 484 | 617 | 0 | 425 | 542 | 1729 | 1756 | 1730 | 1536 | 1561 | 1527 | 486 | 527 | 498 | 406 | 451 | 421 |

Zeros in red are missing data due to machine down time during the run.