

Supplementary Files

Short-Term and Long-Term Carcinogenic Effects of Food Contaminants (4-Hydroxynonenal and Pesticides) On Colorectal Human Cells: Involvement of Genotoxic and Non-Genomic Mechanisms

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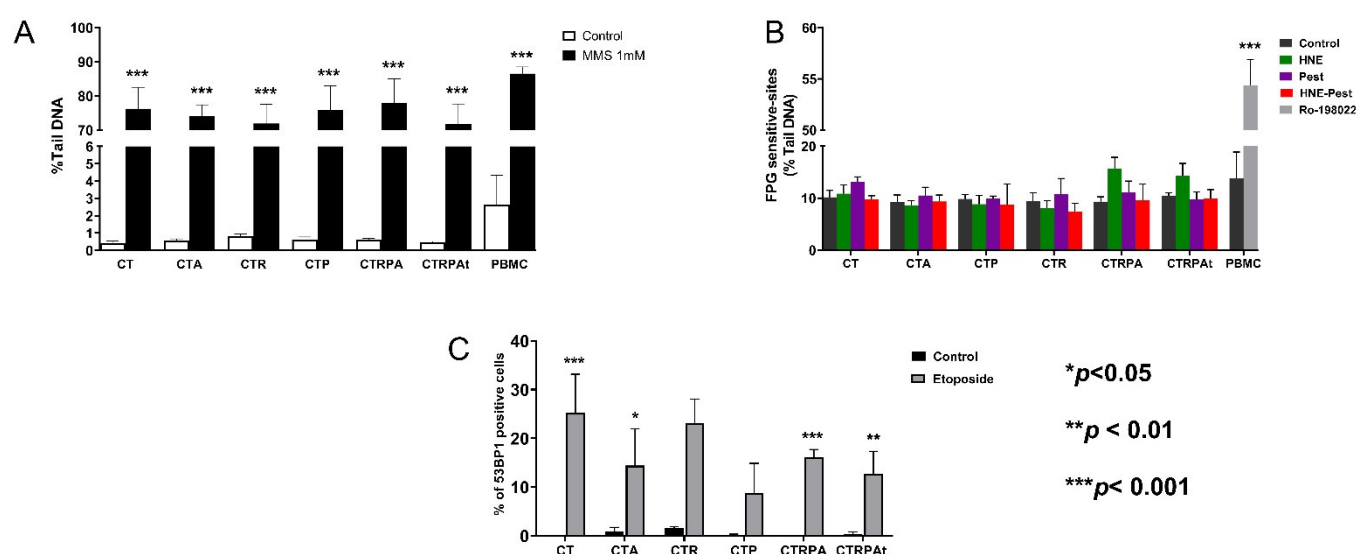


Figure S1. Positive controls for genotoxicity assays. (A) DNA damage evaluated by alkaline comet assay at 2h post-treatment of MMS (positive control) in HCECs and PBMC, an internal standard from a cryopreserved batch as recommended by Minimum Information for Reporting Comet Assay (MIRCA) (Møller et al., 2020). (B) Quantification of Fpg-sensitive sites using Fpg modified comet assay in HCECs at 2h post-treatment and in PBMC treated or not with Ro 19-8022 (positive control). FPG sensitive sites were calculated as described in material and method section. (C) Quantification of HCECs positives for 53BP1 after 2h of treatment to etoposide (positive control). All the results (A, B and C) represent the mean \pm SEM of three independent experiments; statistical differences were analysed by post-hoc tests of a 2-way ANOVA model between control and treated cells (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$).

Cell Titer Glo® Acute exposure		Xcelligence® Acute exposure		Alkaline comet assay Acute exposure	
Effect	P-value	Effect	P-value	Effect	P-value
Cell line	$p < 0.001$ (***)	Cell line	$p = 0.007$ (**)	Cell line	$p = 0.812$ (ns)
Treatment	$p = 0.794$ (ns)	Treatment	$p = 0.995$ (ns)	Treatment	$p < 0.001$ (***)
Cell line*Treatment	$p > 0.999$ (ns)	Cell line*Treatment	$p > 0.999$ (ns)	Cell line*Treatment	$p = 0.997$ (ns)

Fpg-comet assay Acute exposure		53BP1 immunostaining Acute exposure		Counting cells Long-term exposure	
Effect	P-value	Effect	P-value	Effect	P-value
Cell line	$p = 0.762$ (ns)	Cell line	$p = 0.002$ (**)	Cell line	$p < 0.001$ (***)
Treatment	$p < 0.001$ (***)	Treatment	$p < 0.001$ (***)	Treatment	$p < 0.001$ (***)
Cell line*Treatment	$p = 0.994$ (ns)	Cell line*Treatment	$p = 0.021$ (*)	Cell line*Treatment	$p = 0.005$ (**)

Soft agar assay Long-term exposure		γH2AX immunostaining Long-term exposure		53BP1 immunostaining Long-term exposure	
Effect	P-value	Effect	P-value	Effect	P-value
Cell line	$p < 0.001$ (***)	Cell line	$p = 0.301$ (ns)	Cell line	$p = 0.190$ (ns)
Treatment	$p < 0.001$ (***)	Treatment	$p < 0.001$ (***)	Treatment	$p = 0.697$ (ns)
Cell line*Treatment	$p < 0.001$ (***)	Cell line*Treatment	$p = 0.747$ (ns)	Cell line*Treatment	$p = 0.054$ (*)

Basal OCR Seahorse® Long-term exposure		Basal ECAR Seahorse® Long-term exposure		Maximal OCR Seahorse® Long-term exposure	
Effect	P-value	Effect	P-value	Effect	P-value
Cell line	$p = 0.136$ (ns)	Cell line	$p = 0.143$ (ns)	Cell line	$p = 0.013$ (*)
Treatment	$p = 0.003$ (**)	Treatment	$p = 0.003$ (**)	Treatment	$p < 0.001$ (***)
Cell line*Treatment	$p = 0.304$ (ns)	Cell line*Treatment	$p = 0.265$ (ns)	Cell line*Treatment	$p = 0.166$ (ns)

ATP production Seahorse® Long-term exposure		E-cadherin immunoblots Long-term exposure		Vimentin immunoblots Long-term exposure	
Effect	P-value	Effect	P-value	Effect	P-value
Cell line	$p < 0.001$ (***)	Cell line	$p = 0.222$ (ns)	Cell line	$p = 0.818$ (ns)
Treatment	$p = 0.002$ (**)	Treatment	$p = 0.097$ (*)	Treatment	$p = 0.198$ (ns)
Cell line*Treatment	$p = 0.013$ (*)	Cell line*Treatment	$p = 0.833$ (ns)	Cell line*Treatment	$p = 0.915$ (ns)

Figure S2. Summarized tables of statistical analysis.

Cell Titer Glo® Acute exposure		Xcelligence® Acute exposure		Alkaline comet assay Acute exposure	
Effect	P-value	Effect	P-value	Effect	P-value
Cell line	p > 0.999 (ns)	Cell line	p = 0.997 (ns)	Cell line	p = 0.152 (ns)
Treatment	p = 0.794 (ns)	Treatment	p = 0.995 (ns)	Treatment	p < 0.001 (***)
Cell line*Treatment	p > 0.999 (ns)	Cell line*Treatment	p > 0.999 (ns)	Cell line*Treatment	p = 0.997 (ns)
Fpg-comet assay Acute exposure		53BP1 immunostaining Acute exposure		Counting cells Long-term exposure	
Effect	P-value	Effect	P-value	Effect	P-value
Cell line	p = 0.881 (ns)	Cell line	p < 0.001 (***)	Cell line	p = 0.168 (ns)
Treatment	p < 0.001 (***)	Treatment	p < 0.001 (***)	Treatment	p < 0.001 (***)
Cell line*Treatment	p = 0.994 (ns)	Cell line*Treatment	p = 0.021 (*)	Cell line*Treatment	p = 0.326 (ns)
Soft agar assay Long-term exposure		γH2AX immunostaining Long-term exposure		53BP1 immunostaining Long-term exposure	
Effect	P-value	Effect	P-value	Effect	P-value
Cell line	p < 0.001 (***)	Cell line	p = 0.213 (ns)	Cell line	p < 0.001 (***)
Treatment	p < 0.001 (***)	Treatment	p < 0.001 (***)	Treatment	p = 0.658 (ns)
Cell line*Treatment	p < 0.001 (***)	Cell line*Treatment	p = 0.747 (ns)	Cell line*Treatment	p = 0.044 (*)
Basal OCR Seahorse® Long-term exposure		Basal ECAR Seahorse® Long-term exposure		Maximal OCR Seahorse® Long-term exposure	
Effect	P-value	Effect	P-value	Effect	P-value
Cell line	p = 0.142 (ns)	Cell line	p = 0.172 (ns)	Cell line	p = 0.059 (*)
Treatment	p = 0.003 (**)	Treatment	p = 0.003 (**)	Treatment	p < 0.001 (***)
Cell line*Treatment	p = 0.304 (ns)	Cell line*Treatment	p = 0.265 (ns)	Cell line*Treatment	p = 0.166 (ns)
ATP production Seahorse® Long-term exposure		E-cadherin immunoblots Long-term exposure		Vimentin immunoblots Long-term exposure	
Effect	P-value	Effect	P-value	Effect	P-value
Cell line	p < 0.001 (***)	Cell line	p = 0.072 (*)	Cell line	p = 0.053 (*)
Treatment	p = 0.002 (**)	Treatment	p = 0.097 (*)	Treatment	p = 0.198 (ns)
Cell line*Treatment	p = 0.013 (*)	Cell line*Treatment	p = 0.833 (ns)	Cell line*Treatment	p = 0.915 (ns)

Figure S3. Summarized tables of statistical refocused data.

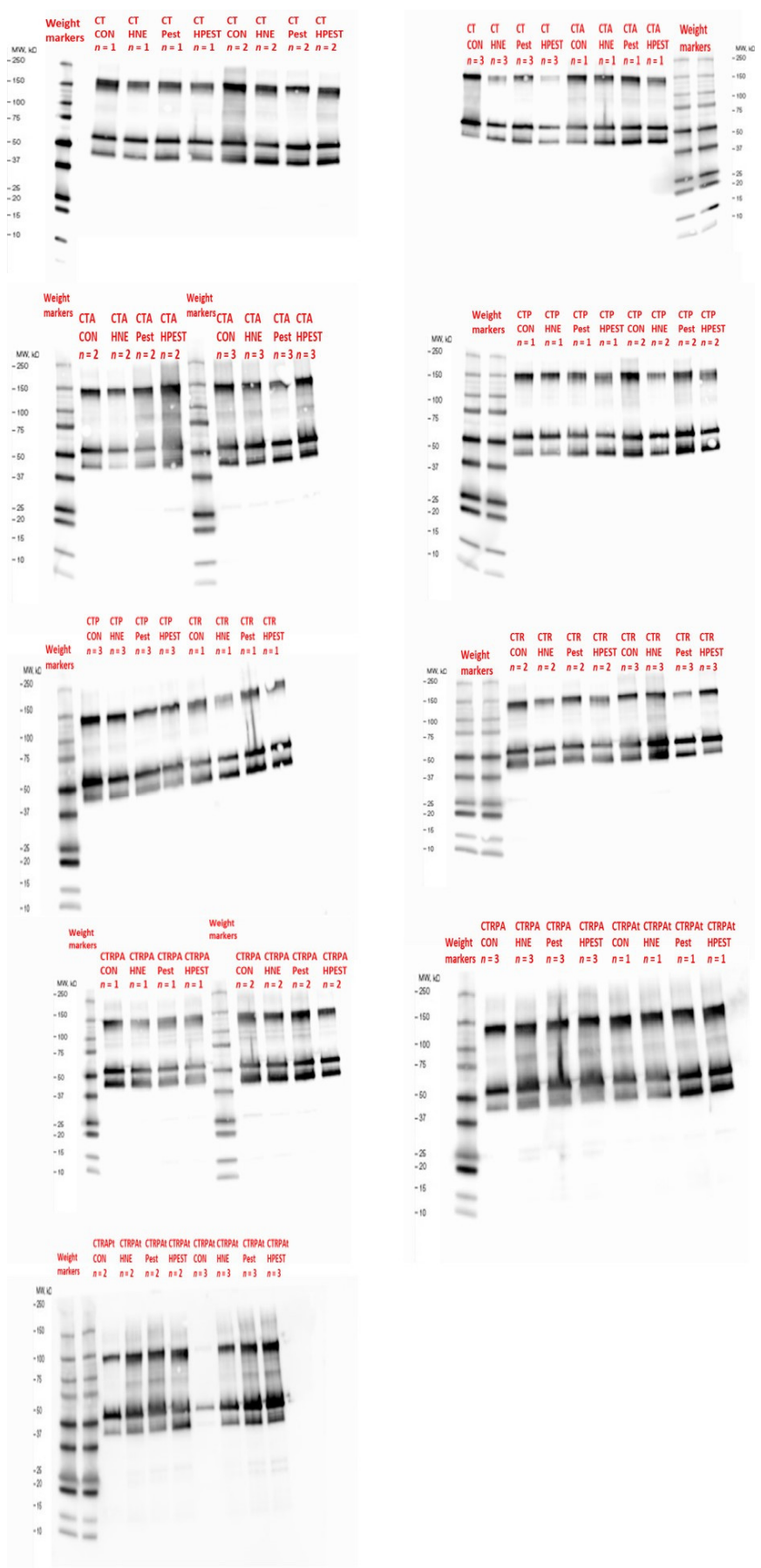


Figure S4. Western blot images.