The insecticides permethrin and chlorpyrifos show limited genotoxicity and no leukemogenic potential in human and murine hematopoietic stem progenitor cells

Virginia C. Rodriguez-Cortez,[1] María Pilar Navarrete-Meneses,[2] Oscar Molina,[1]* Talia Velasco-Hernandez,[1] Jessica Gonzalez,[3] Paola Romecin,[1] Francisco Gutierrez-Agüera,[1] Heleia Roca-Ho,[1] Meritxell Vinyoles,[1] Eric Kowarz,[4] Pedro Marin,[5] Sandra Rodriguez-Perales,[6] Carlos Gomez-Marin,[7] Patricia Perez-Vera,[2] Felipe Cortes-Ledesma,[7] Anna Bigas,[1,3,8] Andrea Terron,[9] Clara Bueno[1,8] and Pablo Menendez[1,8,10]*

[1]Josep Carreras Leukemia Research Institute. Department of Biomedicine. School of Medicine, University of Barcelona. Barcelona, Spain; [2]Laboratorio de Genética y Cáncer, Departamento de Genética Humana, Instituto Nacional de Pediatría, Ciudad de México, México; [3]Cancer Research Program, Institut Hospital del Mar d'Investigacions Mèdiques, Hospital del Mar, Barcelona, Spain; [4]Institute of Pharmaceutical Biology/DCAL, Goethe-University of Frankfurt, Frankfurt/Main, Germany; [5]Hematology Department. Hospital Clínic de Barcelona, Barcelona, Spain; [6]Molecular Cytogenetics Group, Human Cancer Genetics Program, Spanish National Cancer Research Center (CNIO), Madrid, Spain; [7]Topology and DNA Breaks Group, Spanish National Cancer Center (CNIO), Madrid, Spain; [8]Centro de Investigación Biomedica en Red-Oncología (CIBERONC), Madrid, Spain; [9]European Food and Safety Authority. Parma. Italy and [10]Institució Catalana de Recerca i Estudis Avançats (ICREA), Barcelona, Spain.

**MPNM and OM contributed equally.*

Correspondence:

PABLO MENÉNDEZ - pmenendez@carrerasresearch.org

VIRGINIA RODRÍGUEZ-CORTEZ - vrodriguez@carrerasresearch.org

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SUPPLEMENTARY FIGURES

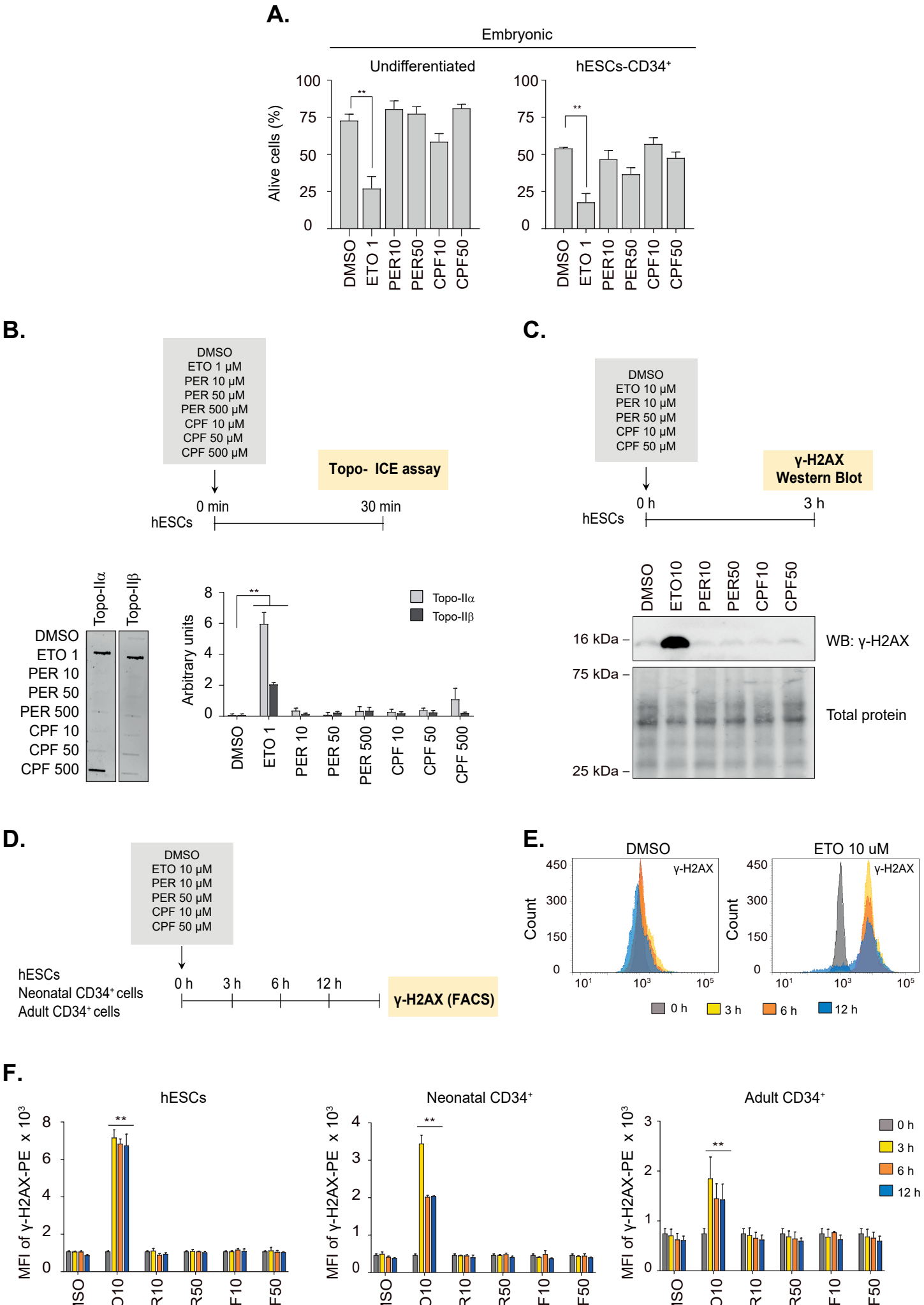
Figure S1. Permethrin and chlorpyrifos are not cytotoxic, are not *bona fide* topo-II poisons and do not affect DNA-DSB formation-repair in embryonic, neonatal or adult human CD34$^+$ HSPCs. A) Viability of hESCs after 24h exposure to etoposide (ETO), permethrin (PER) and chlorpyrifos (CPF). **B)** Upper panel: Experimental design to evaluate the Topo-II poisoning ability of increasing concentrations of PER and CPF in a human topoisomerase *in vivo* complex of enzyme assay (ICE assay). DMSO- and ETO 1 μM-treated hESCs were used as a negative and positive control, respectively. Lower panel: Representative image (left) and quantification (right) of the topo-II isoforms, topo-II α and topo-II β, covalently bound to genomic DNA following 30 min exposure. **C)** Upper panel: Experimental design to analyze the ability of increasing concentrations of PER and CPF to generate DSBs in hESCs, as determined by levels of phosphorylated H2AX at ser139 (γ-H2AX) by western blot. Lower panel: Western blotting of γ-H2AX after the indicated treatments. **D)** Experimental design to analyze the ability of increasing concentrations of PER and CPF to generate and repair DNA DSBs (determined by γ-H2AX by FACS) in hESCs, neonatal and adult CD34$^+$ cells over 12 h. **E)** Representative FACS analysis of γ-H2AX levels on DMSO- and ETO-treated cells.

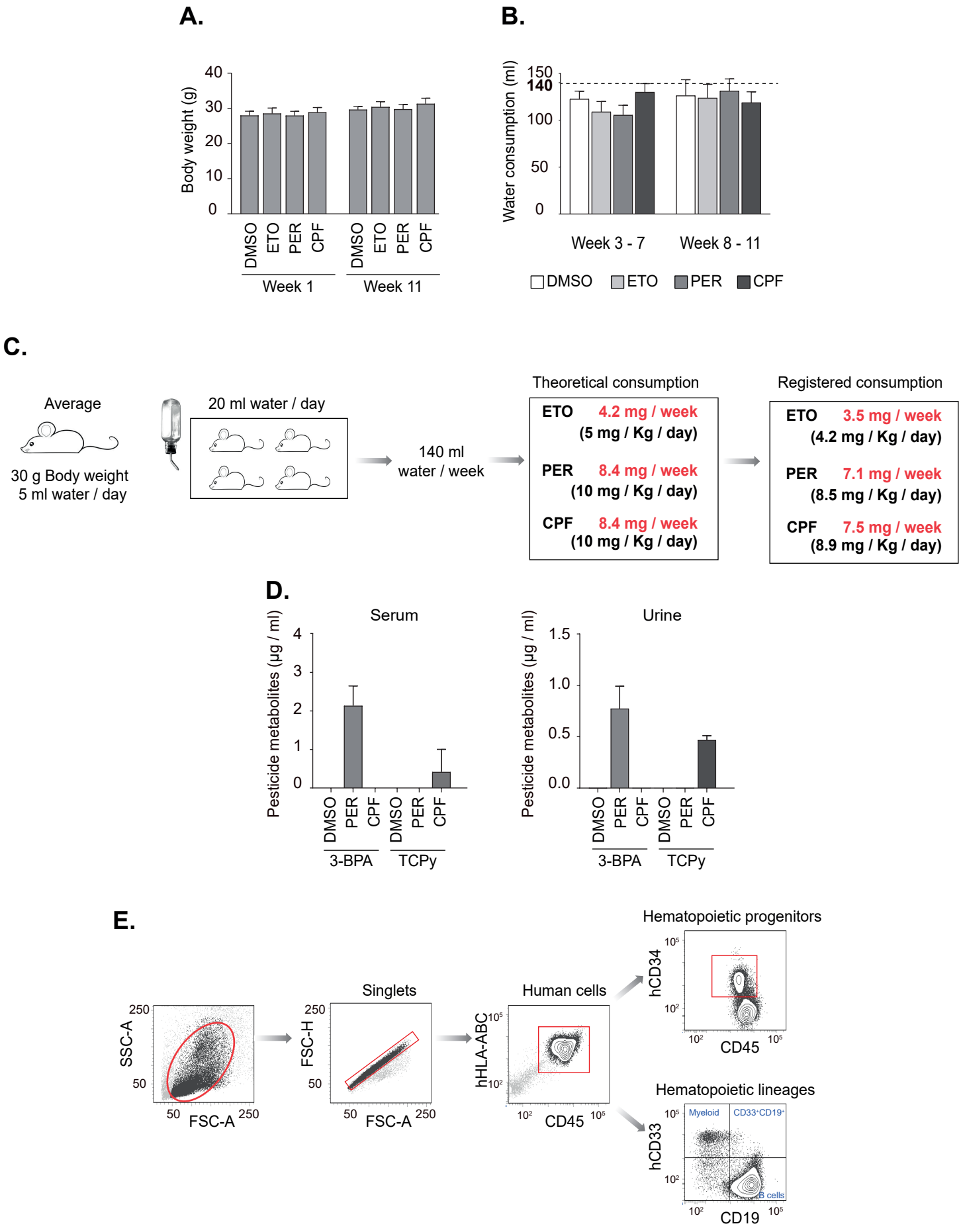
F) Median fluorescence intensity (MFI) of γ-H2AX determined at 0 h, 3 h, 6 h, and 12 h after a single-pulse of the indicated treatments. *p<0.05, **p<0.01 compared with the 0 h time point.

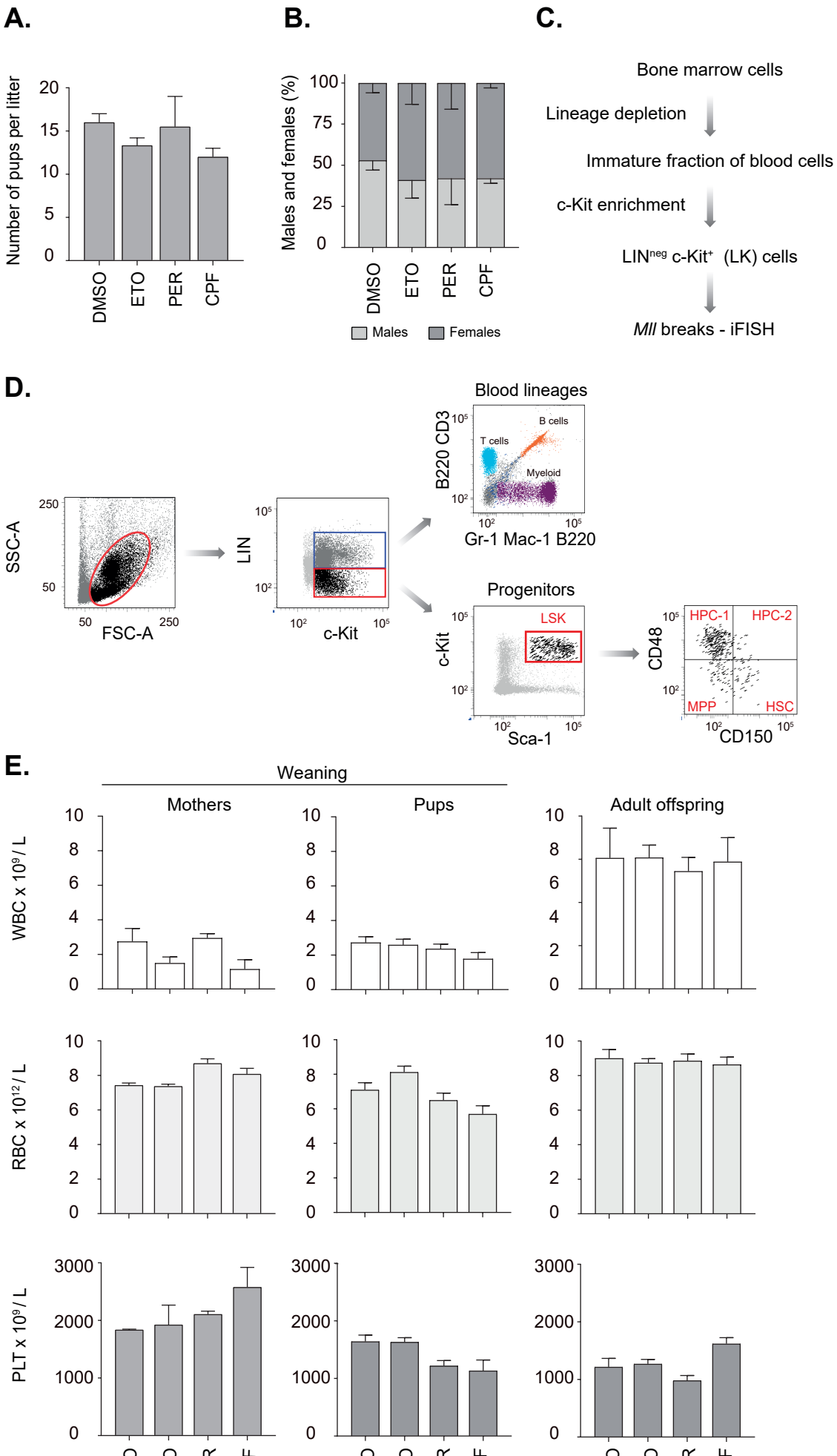
Figure S2. Continuous exposure to permethrin and chlorpyriphos fails to induce *MLL* breaks or leukemia in NSG-reconstituting human CD34$^+$ HSPCs. A) Mice body weight at week 1 and week 11 of the experiment. Each NSG mouse weights an average of 30 g throughout the 12-week experiment. **B)** Actual water consumption per cage at early (3–7) and late (8–11) weeks of the experiment. If each NSG mouse drinks 5 ml of water/day, 140 ml of water consumption are expected per cage per week (the horizontal dotted line). **C)** Cartoon depicting the assumptions used to calculate the theoretical and actual concentration of etoposide, permethrin and chlorpyriphos at which mice were exposed in drinking water. For the target concentration of 10 mg/day/kg (= 0.3 mg/day/mouse) for permethrin and chlorpyriphos, one would expect a consumption of 8.4 mg/cage/week (0.3 mg/day*4mice/cage*7days = 8.4 mg/cage/week). For the target concentration of 5 mg/day/kg (= 0.15 mg/day/mouse) for etoposide, a consumption of 4.2 mg/cage/week (0.15 mg/day*4mice/cage*7days = 4.2 mg/cage/week) is expected. Based on the mice weight and water consumption shown in A and B, the actual intake of permethrin, chlorpyriphos and etoposide was 8.5, 9 and 4.2 mg/kg/day, respectively. **D)** GC-MS quantification of the PER metabolite, 3-phenoxybenzoic acid (3-PBA), and CPF metabolite, 3,5,6-trichloro-2-pyridinol (TCPy), in serum and urine. 3-BPA and TCPy were exclusively detected in mice exposed to permethrin, chlorpyriphos, respectively. **E)** Gating strategy to analyze human hematopoietic reconstitution in NSG mice by FACS. Human cells were identified as CD45$^+$HLA-ABC$^+$. Within this subpopulation, the immature (CD34$^+$CD45$^+$), myeloid (CD33$^+$CD45$^+$) and B-cell lymphoid (CD19$^+$CD45$^+$) graft was analyzed.

Figure S3. Exposure to etoposide, permethrin, and chlorpyriphos during pregnancy. A) Number of pups per litter after pregnancy exposure to the indicated compounds. **B)** Percentage of male and female pups in each treatment group. **C)** Scheme showing the purification steps to isolate Lin$^-$ c-Kit$^+$ (LK cells)

from BM to perform iFISH analysis. **D)** FACS gating strategy to analyze mature hematopoietic cells (myeloid, B and T) from peripheral blood and $Lin^{-}Sca\text{-}1^{+}cKit^{+}$ (LSK) and hematopoietic stem and progenitor cells (HSPCs) subpopulations from bone marrow. From the HSPCs, we analyzed the hematopoietic stem cells (HSCs), multipotent hematopoietic progenitors (MPPs), and restricted hematopoietic progenitor cells 1 and 2 (HPC-1 and HPC-2) subsets. **E)** White blood cell (WBC), red blood cell (RBC) and platelet (PLT) counts in each treatment group in mothers and pups at weaning and in the adult offspring.


A.
Embryonic
Undifferentiated
hESCs-CD34+
Alive cells (%)
100
75
50
25
0
**
DMSO
ETO 1
PER10
PER50
CPF10
CPF50
B.
DMSO
ETO 1 µM
PER 10 µM
PER 50 µM
PER 500 µM
CPF 10 µM
CPF 50 µM
CPF 500 µM
Topo- ICE assay
0 min
30 min
hESCs
Topo-IIα
Topo-IIβ
Arbitrary units
C.
DMSO
ETO 10 µM
PER 10 µM
PER 50 µM
CPF 10 µM
CPF 50 µM
γ-H2AX Western Blot
0 h
3 h
hESCs
ETO10
PER10
PER50
CPF10
CPF50
16 kDa
75 kDa
25 kDa
WB: γ-H2AX
Total protein
D.
DMSO
ETO 10 µM
PER 10 µM
PER 50 µM
CPF 10 µM
CPF 50 µM
hESCs
Neonatal CD34+ cells
Adult CD34+ cells
0 h
3 h
6 h
12 h
γ-H2AX (FACS)
E.
DMSO
ETO 10 uM
γ-H2AX
Count
450
300
150
0
10¹
10³
10⁵
0 h
3 h
6 h
12 h
F.
hESCs
Neonatal CD34+
Adult CD34+
MFI of γ-H2AX-PE x 10³
**
DMSO
ETO10
PER10
PER50
CPF10
CPF50
0 h
3 h
6 h
12 h


A.
Body weight (g)
40
30
20
10
0
DMSO
ETO
PER
CPF
DMSO
ETO
PER
CPF
Week 1
Week 11
B.
Water consumption (ml)
150
140
100
50
0
Week 3 - 7
Week 8 - 11
DMSO
ETO
PER
CPF
C.
Average
30 g Body weight
5 ml water / day
20 ml water / day
140 ml
water / week
Theoretical consumption
ETO 4.2 mg / week
(5 mg / Kg / day)
PER 8.4 mg / week
(10 mg / Kg / day)
CPF 8.4 mg / week
(10 mg / Kg / day)
Registered consumption
ETO 3.5 mg / week
(4.2 mg / Kg / day)
PER 7.1 mg / week
(8.5 mg / Kg / day)
CPF 7.5 mg / week
(8.9 mg / Kg / day)
D.
Serum
Pesticide metabolites (µg / ml)
4
3
2
1
0
DMSO
PER
CPF
DMSO
PER
CPF
3-BPA
TCPy
Urine
Pesticide metabolites (µg / ml)
1.5
1.0
0.5
0.0
DMSO
PER
CPF
DMSO
PER
CPF
3-BPA
TCPy
E.
SSC-A
250
50
50
FSC-A
250
Singlets
FSC-H
250
50
50
FSC-A
250
Human cells
hHLA-ABC
10^5
10^2
10^2
CD45
10^5
Hematopoietic progenitors
hCD34
10^5
10^2
10^2
CD45
10^5
Hematopoietic lineages
hCD33
10^5
10^2
Myeloid
CD33+CD19-
B cells
10^2
CD19
10^5


A.
Number of pups per litter
20
15
10
5
0
DMSO
ETO
PER
CPF
B.
Males and females (%)
100
75
50
25
0
DMSO
ETO
PER
CPF
Males
Females
C.
Bone marrow cells
Lineage depletion
Immature fraction of blood cells
c-Kit enrichment
LIN^neg c-Kit^+ (LK) cells
Mll breaks - iFISH
D.
SSC-A
250
50
50
250
FSC-A
LIN
10^5
10^2
10^2
10^5
c-Kit
Blood lineages
B220 CD3
B cells
T cells
Myeloid
Gr-1 Mac-1 B220
Progenitors
c-Kit
LSK
Sca-1
CD48
HPC-1
HPC-2
MPP
HSC
CD150
E.
Weaning
Mothers
Pups
Adult offspring
WBC x 10^9 / L
RBC x 10^12 / L
PLT x 10^9 / L
10
8
6
4
2
0
3000
2000
1000
0
DMSO
ETO
PER
CPF