Facultad de Medicina
UNIVERSIDAD DE BUENOS AIRES

CEFYBO
CONICET

PRIMER CICLO DE CONFERENCIAS DE LA PRIMERA CÁTEDRA DE TOXICOLOGÍA

Hidrocarburos poliaromáticos

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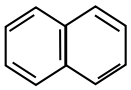
Universidad de Buenos Aires

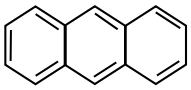
Hidrocarburos poliaromáticos (PAHs)

- PAHs no halogenados
- PAHs halogenados

PAHs no halogenados

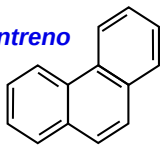
Naftaleno





Antraceno

Fenantreno

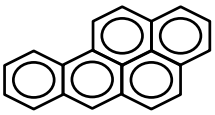


PAHs halogenados

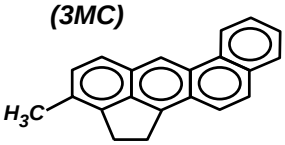
- Dibenzo-p-dioxinas policlorados (dioxinas)
- Bifenilos policlorados (PCBs)
- Dibenzofuranos policlorados (PCDFs)
- Bifenilos polibromados (PBBs)
- Difeniléteres policlorados (PCDEs)
- Difeniléteres polibromados (PBDEs)

PAHs no halogenados

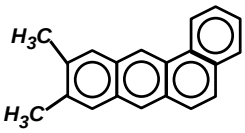
Benzo[a]pireno
(BaP)



3-Metilcolantreno
(3MC)



9,10-Dimetilbenzoantraceno
(DMBA)



PAHs no halogenados

Fuentes

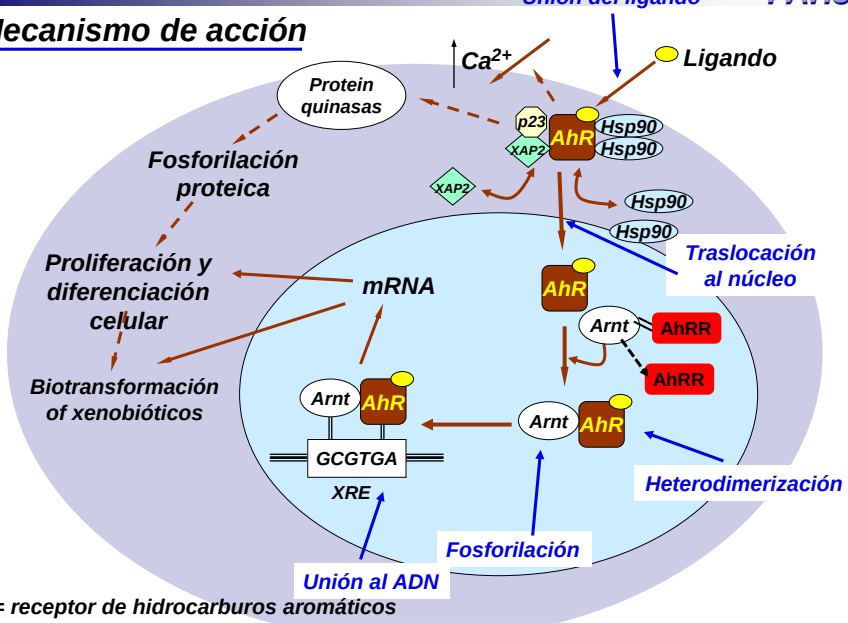
Contaminantes ambientales

Combustión incompleta de

- Madera
- Carbón
- Motor de vehículos
- Pirólisis de aminoácidos, lípidos o carbohidratos
- Tabaco
- Incienso

PAHs

Mecanismo de acción



AhR = receptor de hidrocarburos aromáticos
XRE = elemento de respuesta al xenobiótico
Arnt = traslocador nuclear del receptor de hidrocarburos aromáticos

PAHs

Efectos

- Toxicidad cutánea → Cloracné
- Disruptor endócrino
- Toxicidad hepática

→ Hipertrofia, Hiperplasia, Fibrosis, Inflamación, Acumulación de lípidos

- Alteraciones metabólicas: porfiria, diabetes, estrés oxidativo
- Inducción enzimática (CYPs, GST)
- Toxicidad reproductiva y del desarrollo
- Alteraciones en el tracto gastrointestinal
- Neurotoxicidad en niños
- Inmunotoxicidad (infecciones, atrofia del timo, linfocitos T alterados

IARC: carcinógeno (promotor de tumores en distintos tejidos)

(Agencia Internacional para la Investigación del Cáncer)

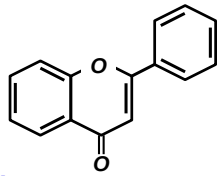
Rol del AhR

- Respuestas adaptativas (biotransformación de xenobióticos)
- Respuestas tóxicas
- Organogénesis
- Funciones hormonales

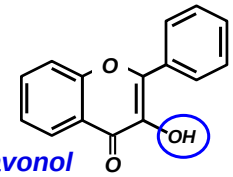
Genes blanco de acción de los AhR

- Enzimas de biotransformación (Cyp1A1, Cyp1A2, Cyp1B1)
- Enzimas de conjugación (UDP-G-transferasa, GST-S-transferasa)
- Genes reguladoras del ciclo celular (p27, jun-B)
- Genes reguladoras de la apoptosis (bax, bcl-2)

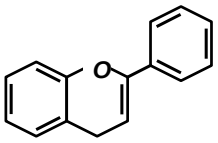
Flavonoides



Flavona



Flavonol



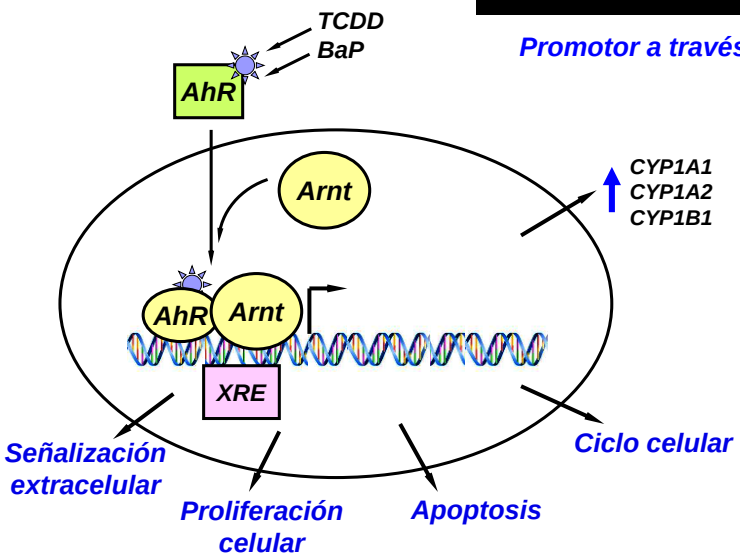
Compuestos fenólicos (>5000)

- Alimentos no energéticos
- Naturales o sintéticos

- Antioxidantes
- Neutralizan radicales libres
- Quelan Fe, Cu, Zn

Efecto carcinógeno

Promotor a través de AhR



Humo del cigarrillo

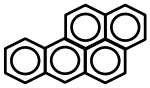
Fumadores →

- ⇩ Volumen semen
- ⇩ Recuento espermático
- ⇩ Motilidad espermática
- ⇩ Viabilidad de las células germinales
- ⇧ Defectos estructurales células germinales
- ⇩ Tasa de fertilidad
- ⇩ Tasa de implantación
- ⇧ Niveles de ROS
- ⇩ Niveles de GSH, actividad de SOD
- ⇧ Aductos ADN en espermatozoides

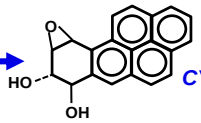
Benzo[a]pireno (BaP)

Exposición aguda

7 días
25 días
60 días

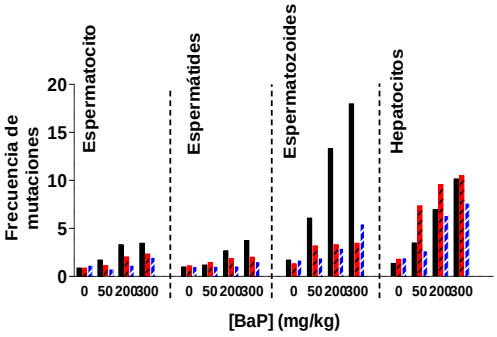


Benzo[a]pireno

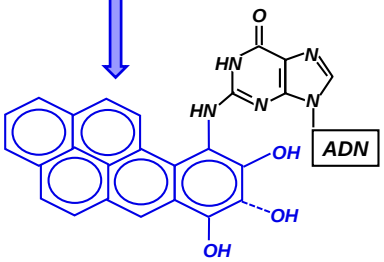


CYP1A1

BaP-7,8-diol-9,10-epoxido



ADN

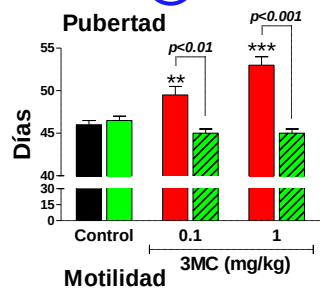


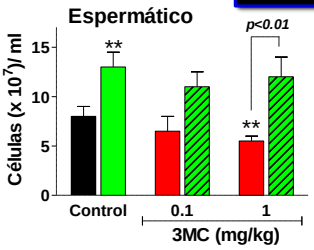
Aductos en espermatozoides

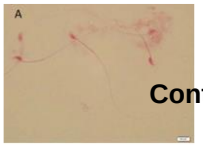
Xu y col. Plos One 2014

3-Metilcolantreno (3MC)

Exposición repetitiva



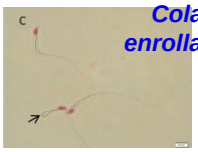




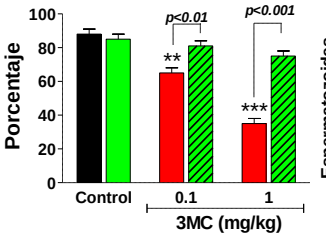
Control

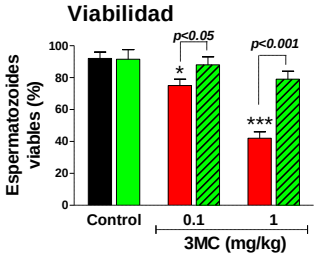
BaP (50 mg/kg)

Cabeza anormal



Cola enrollada

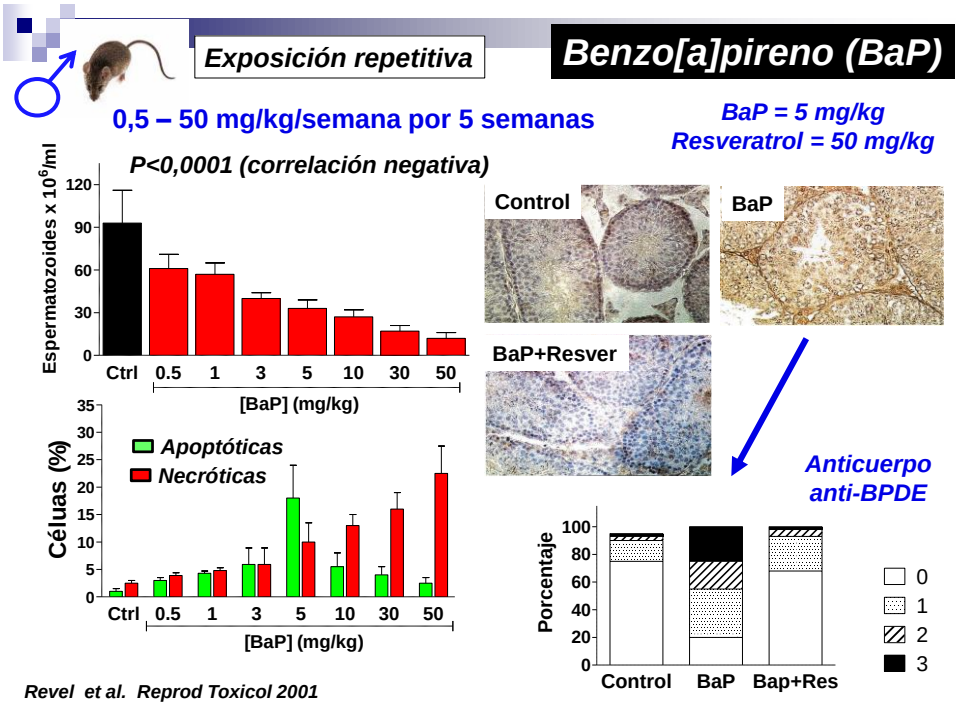




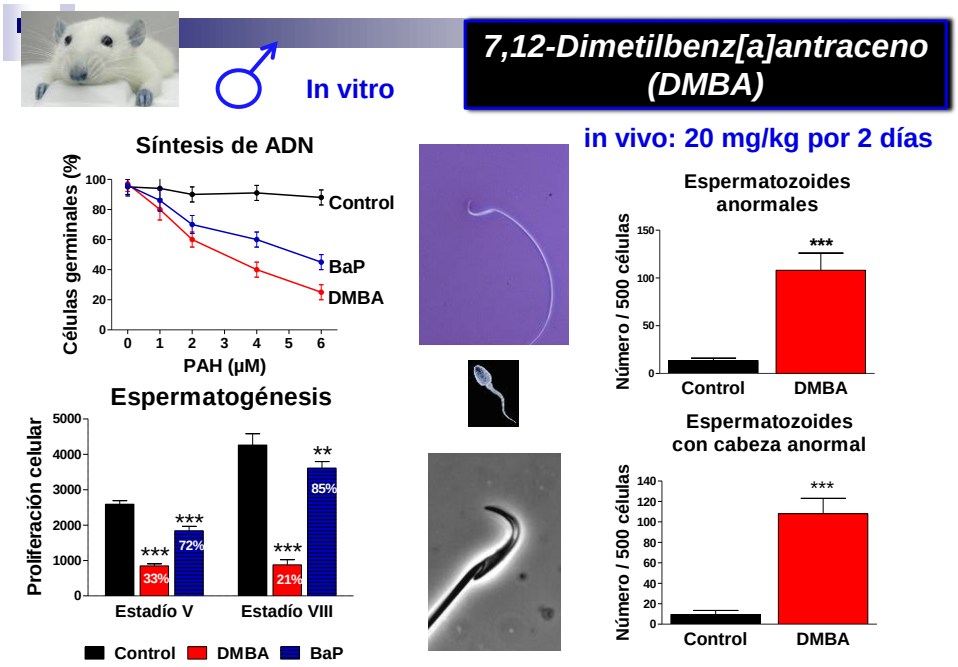
Ctrl αNF (80 mg/kg) 3MC 3MC + αNF

Faletti et al. No publicado aún

Jeng et al. Enrivor Toxicol 2013



Revel et al. Reprod Toxicol 2001



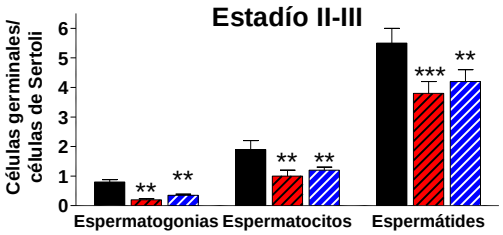
Georgellis et al. Mutat Res 1990

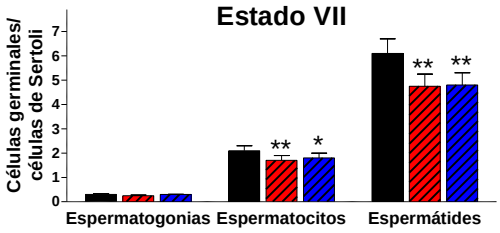
Raj et al. Mutat Res 1984

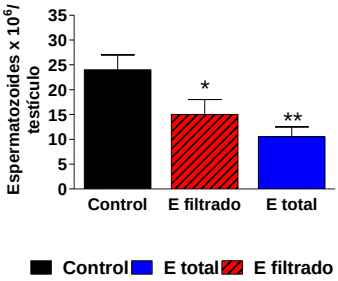
Escape de motores diésel

Exposición crónica

Exposición diaria
Nacimiento → 3 meses
6 h / día, 5 días/semana



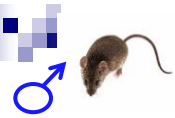




⇧ [E2] ⇩ FSH
⇧ [T] ⇩ LH

Watanabe y Oonuki Environ Health Perspect 1999

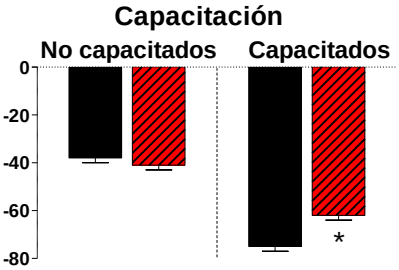
Humo del cigarrillo

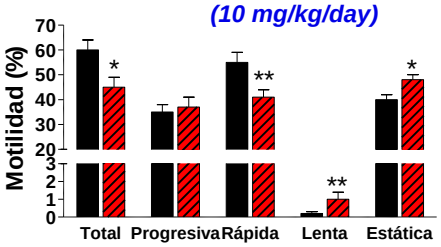


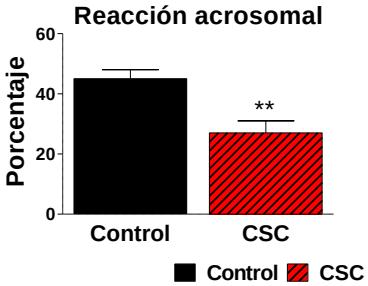
Exposición diaria (40 días)

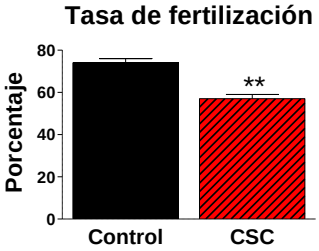
(CSC)

(10 mg/kg/day)

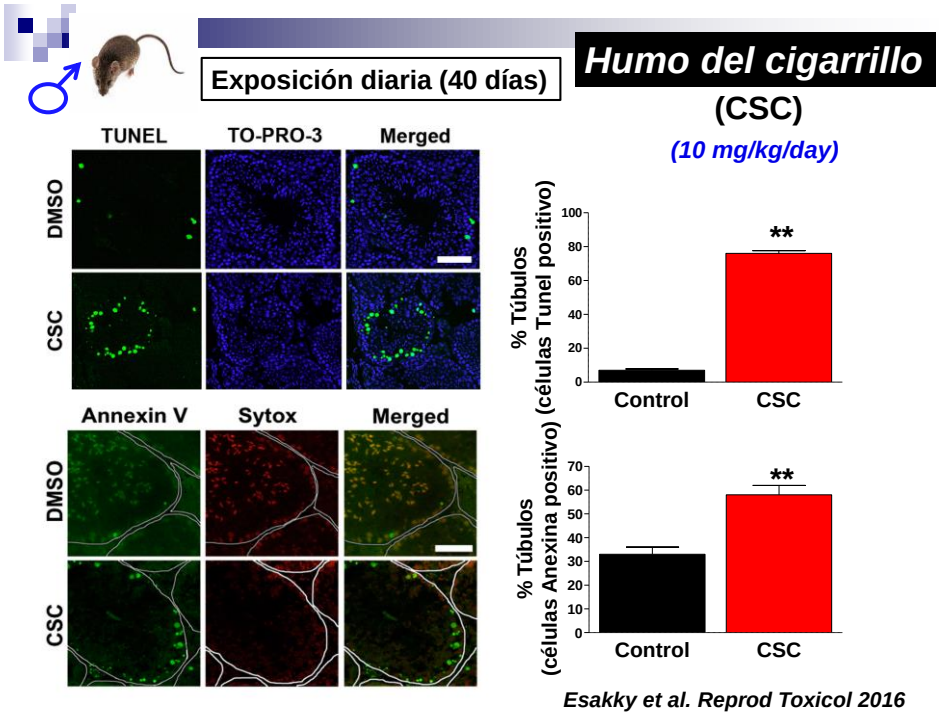




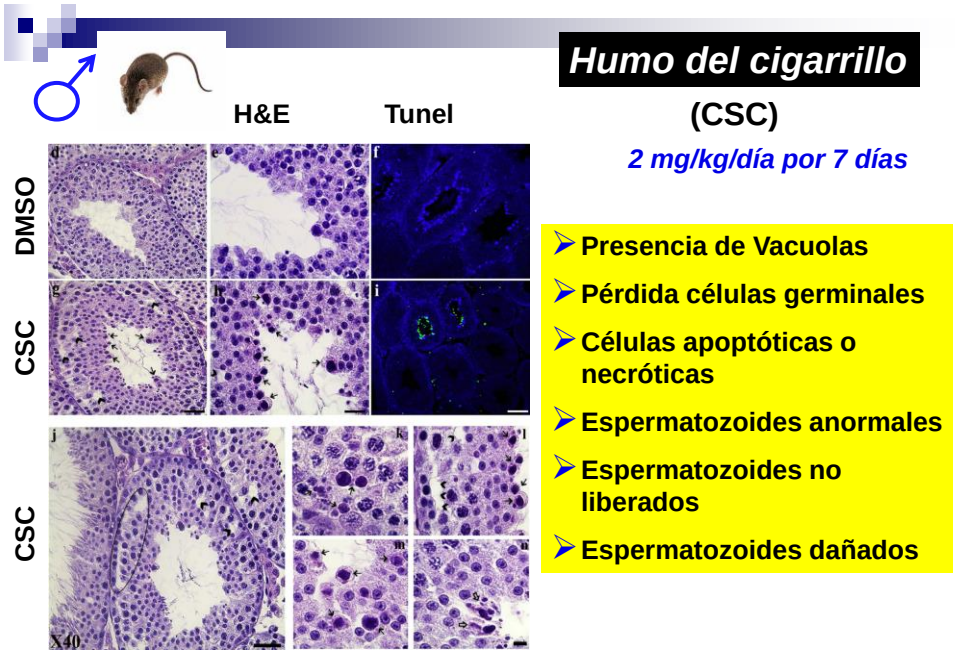




Esakky et al. Reprod Toxicol 2016



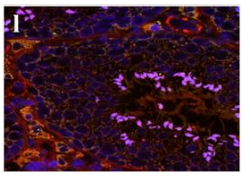
Esakky et al. Reprod Toxicol 2016



- Presencia de Vacuolas
- Pérdida células germinales
- Células apoptóticas o necróticas
- Espermatozoides anormales
- Espermatozoides no liberados
- Espermatozoides dañados

Esakky et al. Reprod Toxicol. 2012

Control

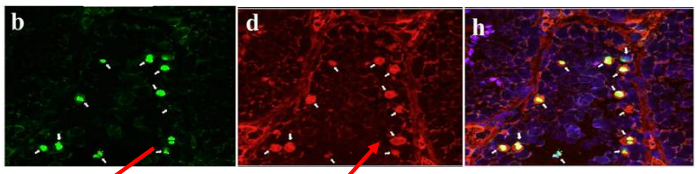


Humo del cigarrillo

(CSC)

2 mg/kg/día por 7 días

Tunel | **Anti-BPDE** | ***Fusión***



Células apoptóticas

Células con aductos en el ADN

Presencia del BaP

Esakky et al. Reprod Toxicol. 2012

En resumen

Modelo animales →

PAHs

- ⇧ Células germinales anormales
- ⇩ Espermatogénesis
- ⇧ Apoptosis de células germinales
- ⇧ Frecuencia de mutaciones
- Induce necrosis
- Induce desbalance hormonal
- Atrasa la pubertad
- ⇩ Proliferación celular
- ⇩ Motilidad espermática
- ⇩ Fertilidad

PAHs y Sistema reproductor femenino

Humo del cigarrillo

♀

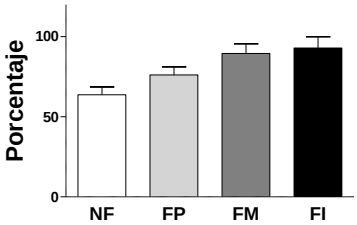
Fumadores ➡

- Menopausia temprana
- Pérdida de folículos inmaduros
- Desarrollo folicular anormal
- Inhibición de la maduración del oocito
- ⇧ Formación de ROS
- ⇩ Tasa de fertilización
- ⇩ Tasa de implantación
- ⇩ Tasa de embarazo en FIV
- ⇩ Contenido de GSH
- ⇧ Peroxidación lipídica
- Alteraciones en el balance redox

Humo del cigarrillo

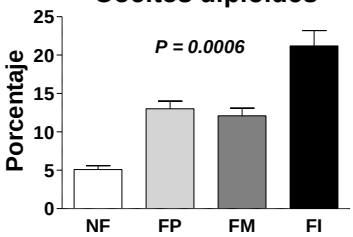
Oocito diploide
46 cromosomas en metafase II

Tasa de fertilización



$x^2 = 9.32$, P = 0.025
Z = 2.635, P = 0.008

Oocitos diploides



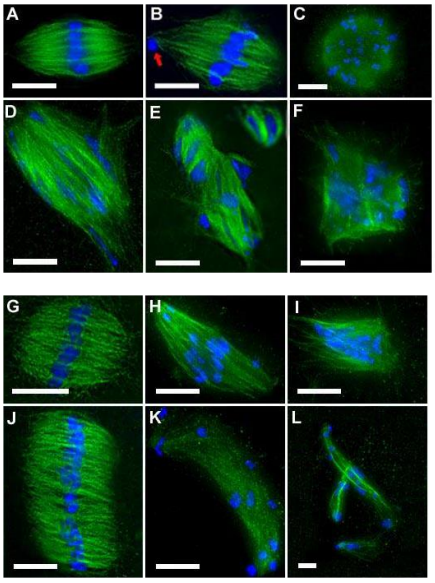
Retraso en la maduración de oocitos

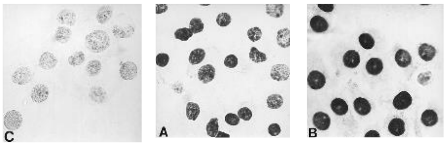
Fallas probables en la meiosis

Zenzes et al. Hum Reprod 1995

Humo del cigarrillo

Aductos ADN en células de granulosa





No fumadora *Fumadora* *Fumadora pasiva*

Altera el huso acromático

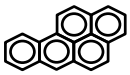
Altera la meiosis

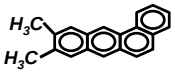
Forma aductos ADN en oocitos

Zenzes et al. 1998, 2000, 2004

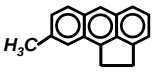
PAHs no halogenados

Benzo[a]pireno (BaP)





9,10-Dimetilbenzoantraceno (DMBA)

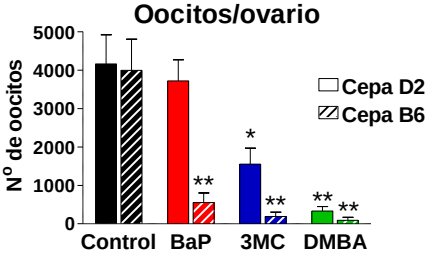


3-Metilcolantreno (3MC)

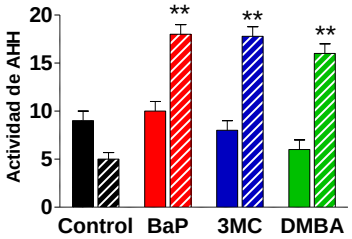
♀ ⬇ *Dosis aguda (80 mg/kg)*

Destrucción de los folículos primordiales

Oocitos/ovario



Activiadad enzimática de AHH ovárica

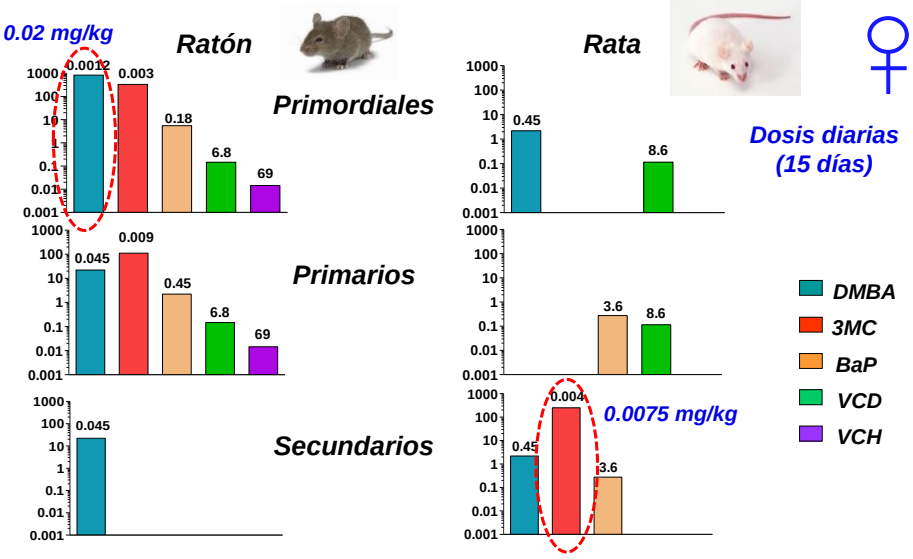


DMBA > 3MC > BaP

Mattison et al. Toxicol Appl Pharmacol, 1980

ÍNDICE DE OVOTOXICIDAD — PAHs

Menor dosis del tóxico que produce la pérdida del 50% de los folículos ováricos

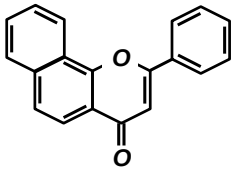


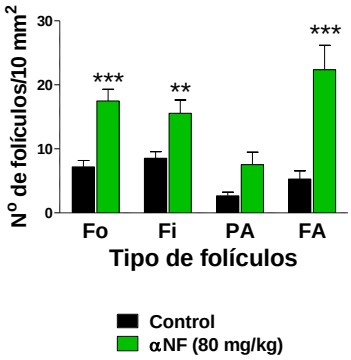
Borman et al. Toxicol App Pharmacol, 2000

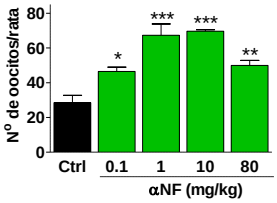
A-Naftoflavona (αNF)

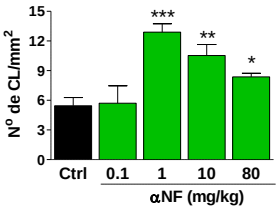
Exposición diaria (prepúber)

7,8-Benzoflavona









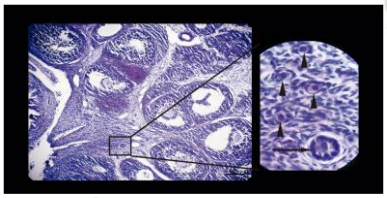
Estimula el desarrollo folicular
Estimula la tasa ovulatoria
Aumento de la reserva folicular????

Barreiro et al, Toxicol Appl Pharmacol, 252: 11, 2011

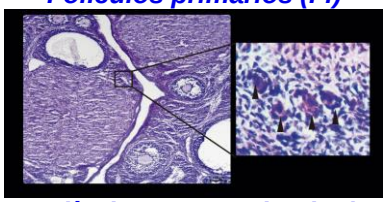
3-Metilcolantreno (3MC)

Exposición diaria (prepúber)

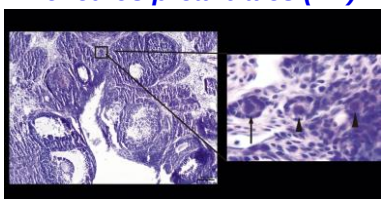
Folículos primordiales Fo

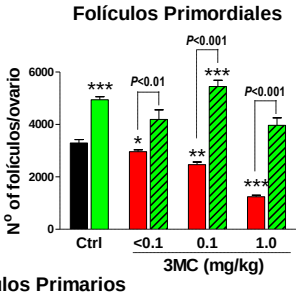


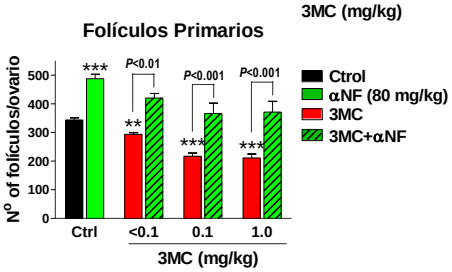
Folículos primarios (Fi)



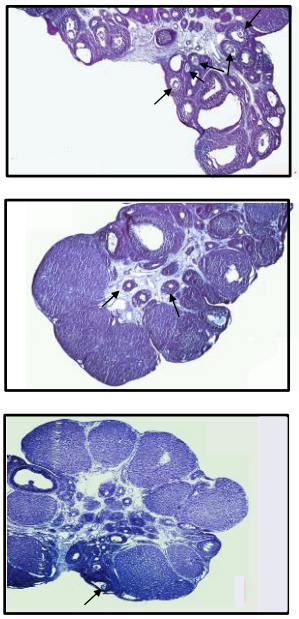
Folículos preantrales (PA)







Rhon-Calderón et al, Toxicology, 353-354, 58, 2016

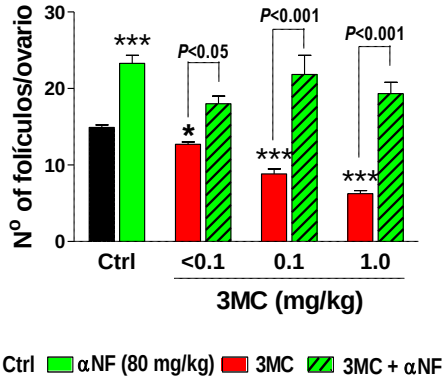


♀

3-Metilcolantreno (3MC)

Exposición diaria (prepúber)

Folículos Antrales

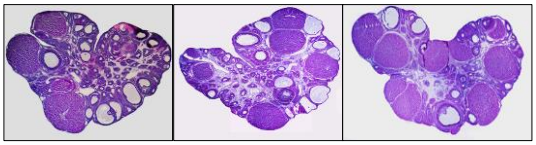


Rhon-Calderón et al, Toxicology, 353-354, 58, 2016

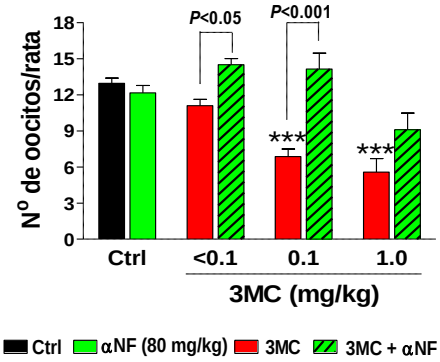
Exposición diaria (prepúber)

♀

3-Metilcolantreno (3MC)



Tasa ovulatoria



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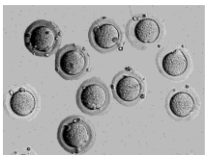
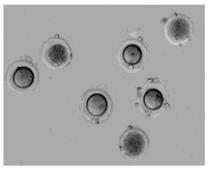
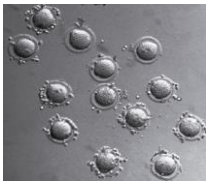


3MC 1 mg/kg



3MC+αNF



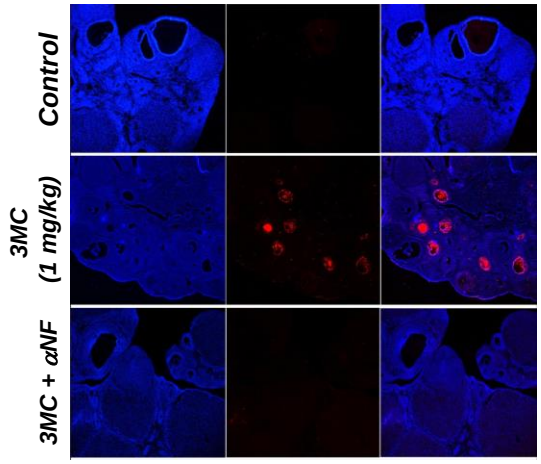
Rhon-Calderón et al, Toxicology, 353-354, 58, 2016

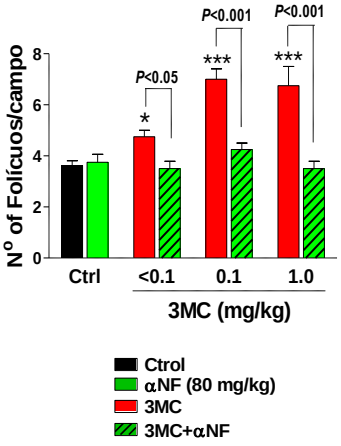
3-Metilcolantreno (3MC)

♀

Exposición diaria (prepúber)

Folículos antrales apoptóticos

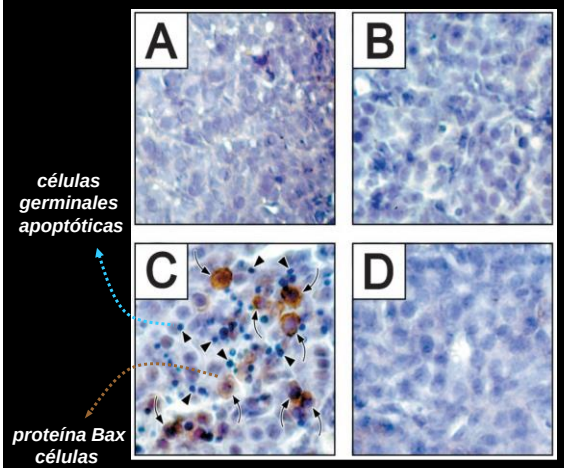




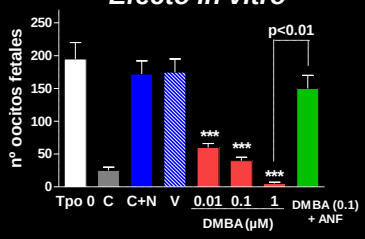
Rhon Calderón et al. Enviado a Arch Toxicol 2019

PAHs

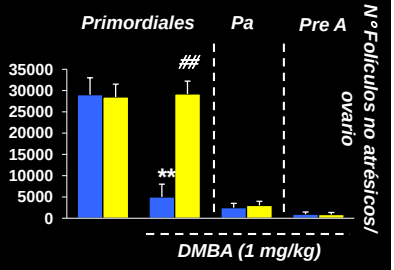
Efecto de DMBA sobre células germinales de ovario fetal



Efecto in vitro



Efecto in vivo

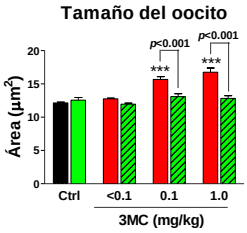


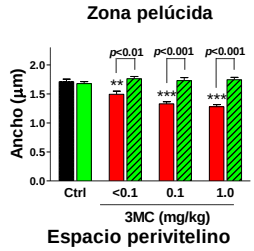
Matikainen et al, Endocrinology, 143: 615, 2002

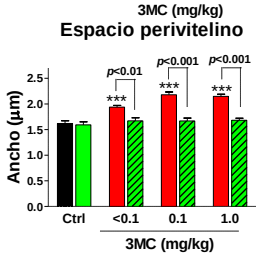
Integridad del oocito

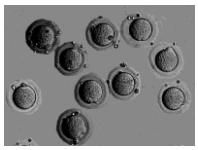
♀ **3-Metilcolantreno (3MC)**

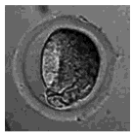
Exposición diaria (prepúber)

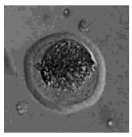


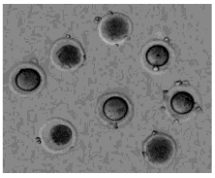










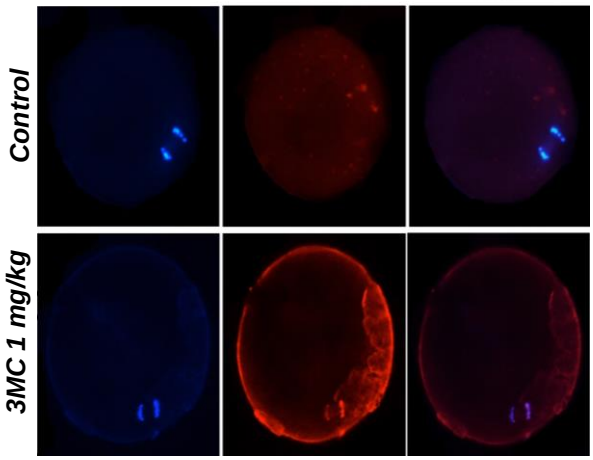


Ctrl αNF (80 mg/kg) 3MC 3MC + αNF

Rhon Calderón et al. Enviado a Arch Toxicol 2019

Viabilidad del oocito

♀ **3-Metilcolantreno (3MC)**



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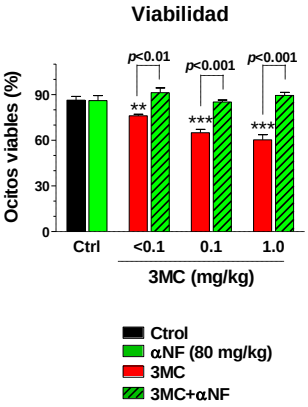
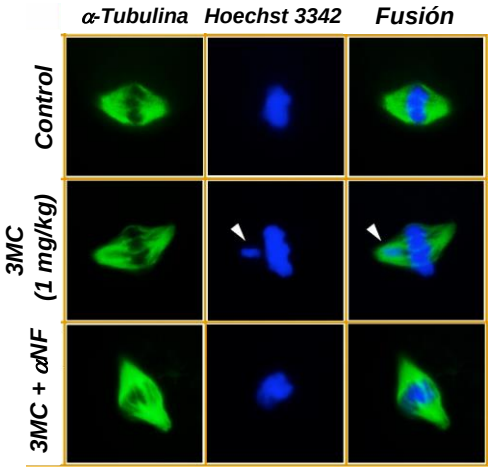


Rhon Calderón et al. Enviado a Arch Toxicol 2019

3-Metilcolantreno (3MC)

♀

Segregación meiótica



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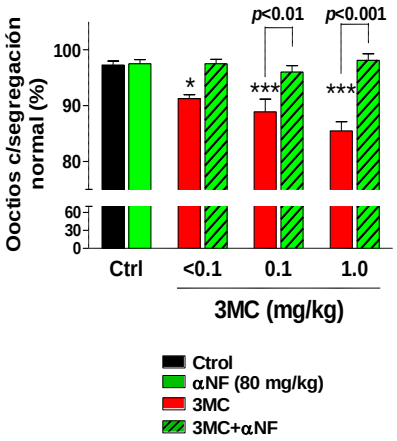


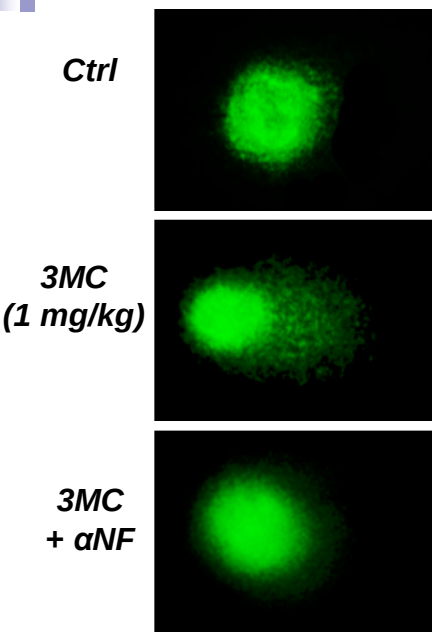
Rhon Calderón et al. Enviado a Arch Toxicol 2019

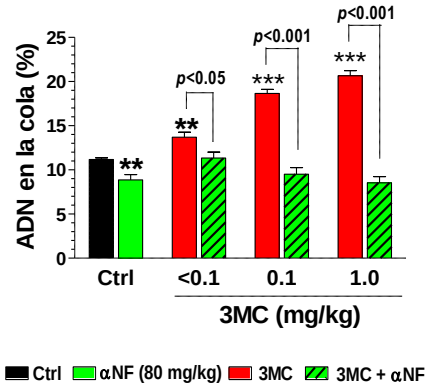
3-Metilcolantreno

♀

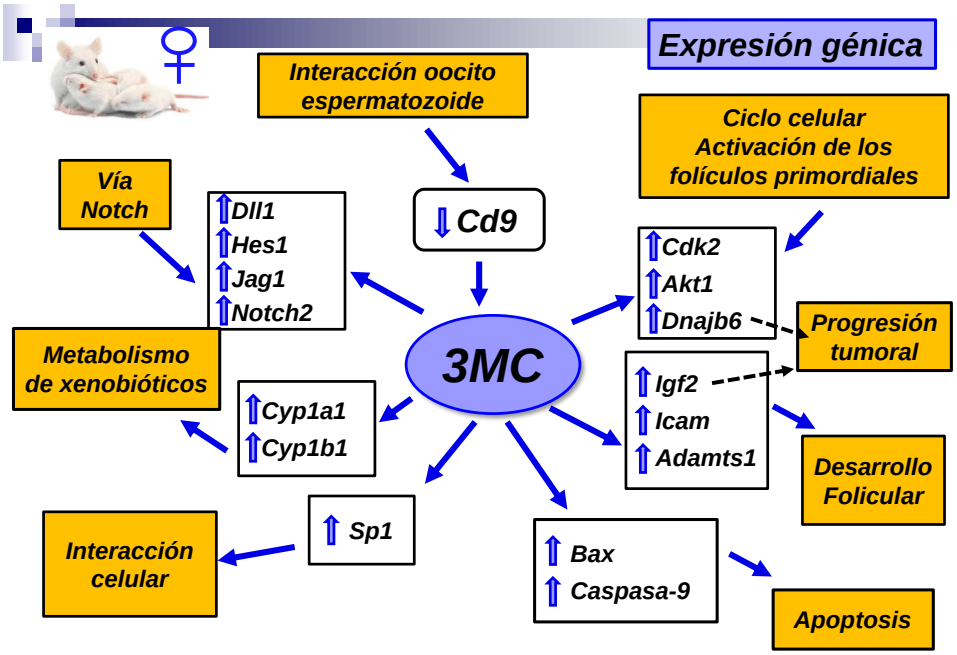
Daño al ADN

Oocito

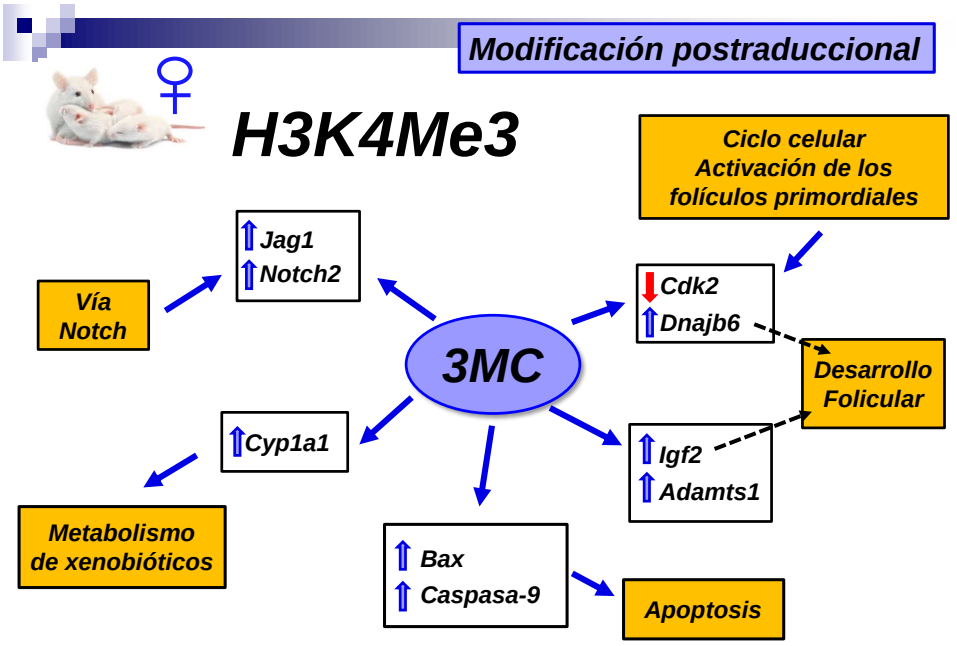




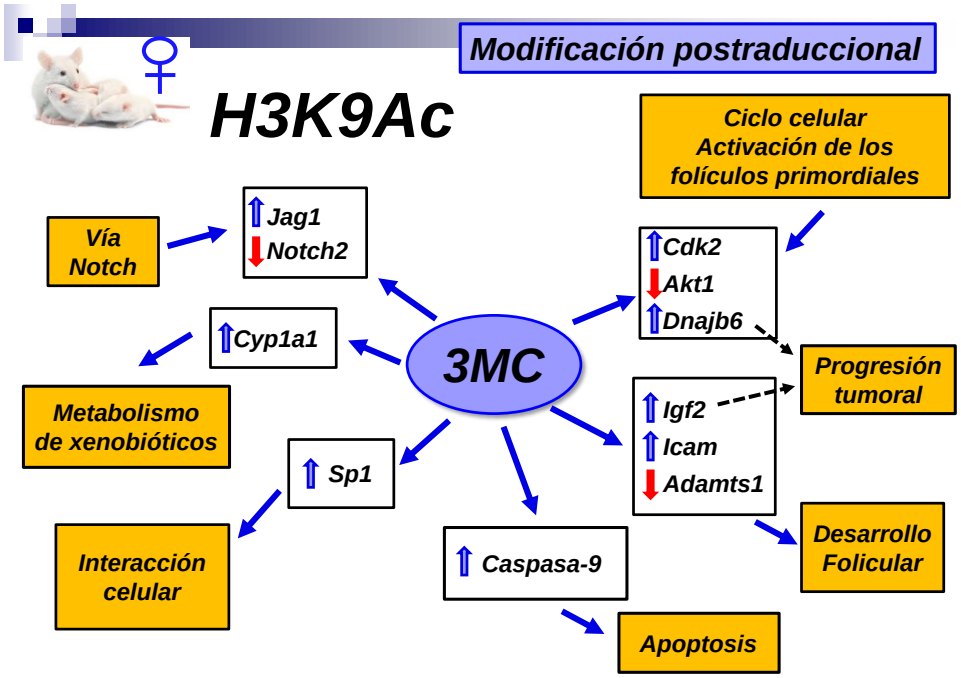
Rhon-Calderón et al. Toxicology 2016



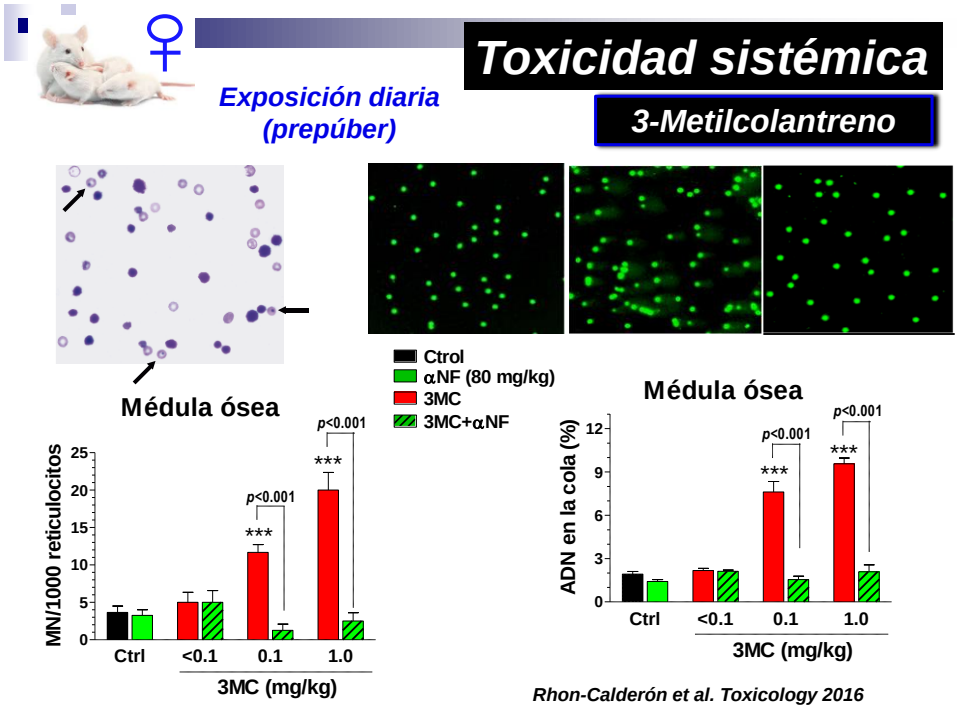
Rhon-Calderón et al. Arch Toxicol 2018



Rhon-Calderón et al. Arch Toxicol 2018



Rhon-Calderón et al. Arch Toxicol 2018



Rhon-Calderón et al. Toxicology 2016

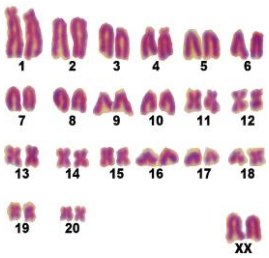
Toxicidad sistémica

♀

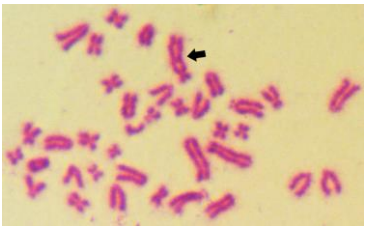
3-Metilcolantreno

Aberraciones cromosómicas

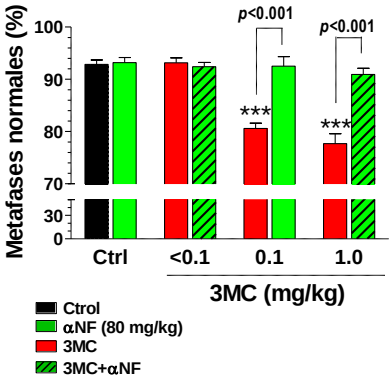
Cariotipo control



Cariotipo 3MC (1 mg/kg)



Exposición diaria (prepúber)

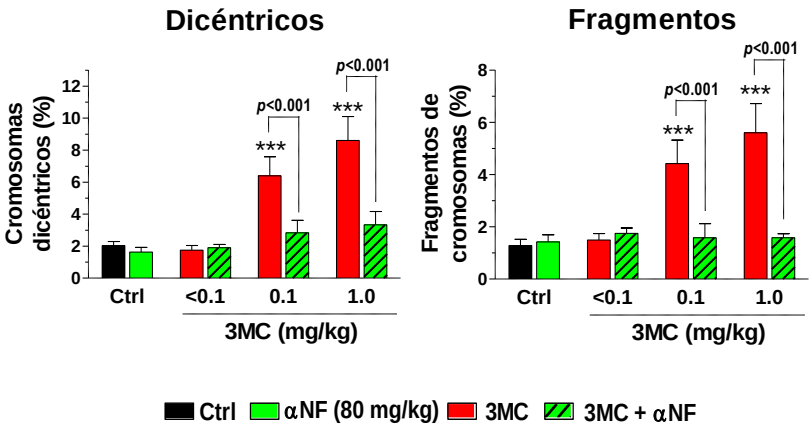


Rhon Calderón et al. Enviado a Arch Toxicol 2019

♀

3-Metilcolantreno (3MC)

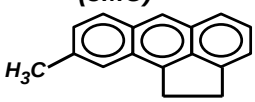
Aberraciones cromosómicas



Rhon Calderón et al. Enviado a Arch Toxicol 2019

Conclusiones

3-Metilcolantreno (3MC)



Exposiciones repetitivas →

Diarias (0,1-1,0 mg/kg)	3 vs/semana (0,1 mg/kg)

Destruye los folículos inmaduros y altera el desarrollo folicular

Afecta la integridad de los oocitos

Altera la expresión de genes involucrados en la función ovárica

Causa efecto sistémico	No causa efecto sistémico

αNF previene el daño sistémico y gonadal causado por 3MC

En resumen

PAHs

Modelo animales →

- ⇧ Células germinales anormales
- Destrucción folículos primordiales
- ⇧ Daño ADN en oocitos
- ⇩ Desarrollo folicular
- ⇩ Ovulación
- ⇧ Frecuencia de aductos de ADN
- ⇧ Aberraciones cromosómicas
- Induce apoptosis
- Induce desbalance hormonal
- Altera regulación genética y epigenética
- ⇩ Atrasa la pubertad
- ⇩ Fertilidad

Conclusiones

Bajas dosis de exposición repetitiva a PAHs causan toxicidad gonadal sin generar toxicidad sistémica

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Muchas gracias

National Primate Research Center, OHSU, Beaverton, Oregon, USA

Dr. Alejandro Lomniczi
Dr. Carlos A. Toro

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NIH 5R01HD084542-02 (National Institute of Health)