Cr(VI) Toxicity Targets p53-SIRT1-mediated germ cell death that Leads to Premature Ovarian Failure.

Sakhila K. Banu

T3: TEXAS A&M TRIADS FOR TRANSFORMATION
A President's Excellence Fund Initiative

Abstract

Environmental contamination with hexavalent chromium (CrVI) has been increasing in the United States as well as in the developing countries. Due to increased usage of Cr and improper disposal of Cr wastes from the industries, CrVI levels in the environment have been continuously increasing. Exposure to CrVI predisposes human population to various diseases, including cancer, infertility, and developmental problems in children. Findings from our laboratory reported that prenatal exposure to CrVI caused premature ovarian failure through p53-mediated mechanisms. Sirtuin 1 (SIRT1) is an NAD^+-dependent histone deacetylase class III. SIRT1 deacetylates several histones and non-histone proteins including p53. Current study determines a role for SIRT1-p53 network in CrVI-induced apoptosis in the ovary and establishes physical interaction between SIRT1 and p53. Adult pregnant dams were given regular drinking water or CrVI (5 ppm potassium dichromate in drinking water, *ad libitum*), and treated with SIRT1 inhibitor, EX-527 (50 mg/kg body weight, *i.p.,*), during 9.5 -14.5 *days post-coitum*. On postnatal day-1, ovaries from F1 offspring were collected for various analyses. Results indicated that CrVI increased germ cell and somatic cell apoptosis, upregulated acetyl-p53, activated the apoptotic pathway, and inhibited cell survival pathway. CrVI decreased acetyl-p53-SIRT1 co-localization and inhibited the physical interaction between SIRT1 and acetyl-p53 by altering the p53:SIRT1 ratio. EX-527 exacerbated CrVI-induced mechanisms. CrVI also disrupts chromosome segregation during oocyte meiosis at Metaphase-II. The current study for the first time shows a novel mechanism for CrVI-induced apoptosis in the oocytes, mediated through the p53-SIRT1 network. The data obtained through the T3 fund are developed into NIH grants, that were already submitted and in progress.

- Epidemiological evidence from China, Russia, India, Bangladesh, Denmark and Finland.
- Reports from Hinkley, CA
- Remy et al, 2017; Environmental CrVI exposure - A two-generation study from Willits, CA – Infertility in females and developmental problems in children.

Cr6+ Health & Safety Alert
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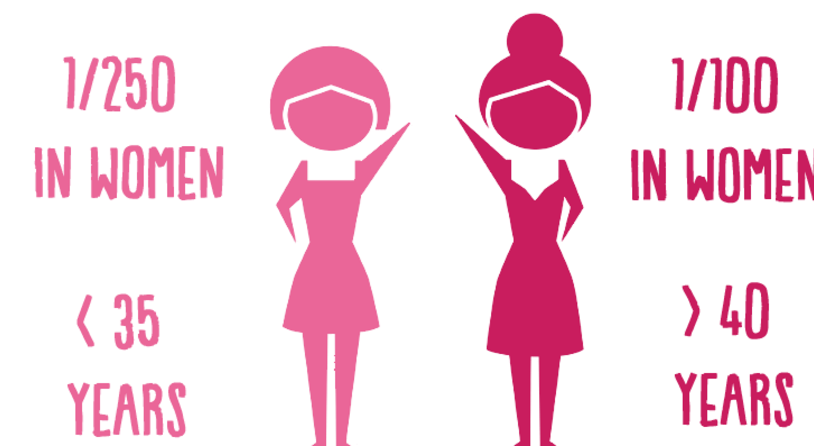
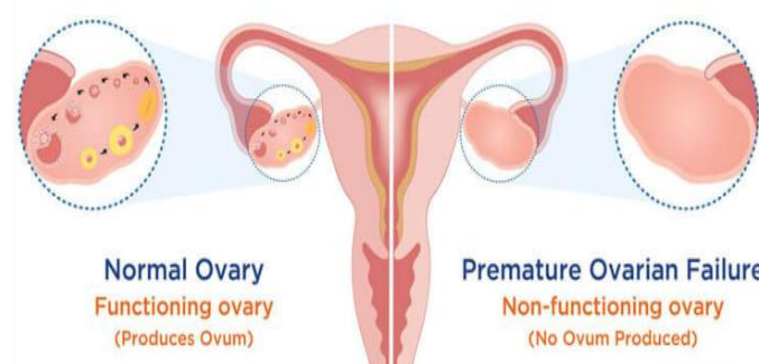
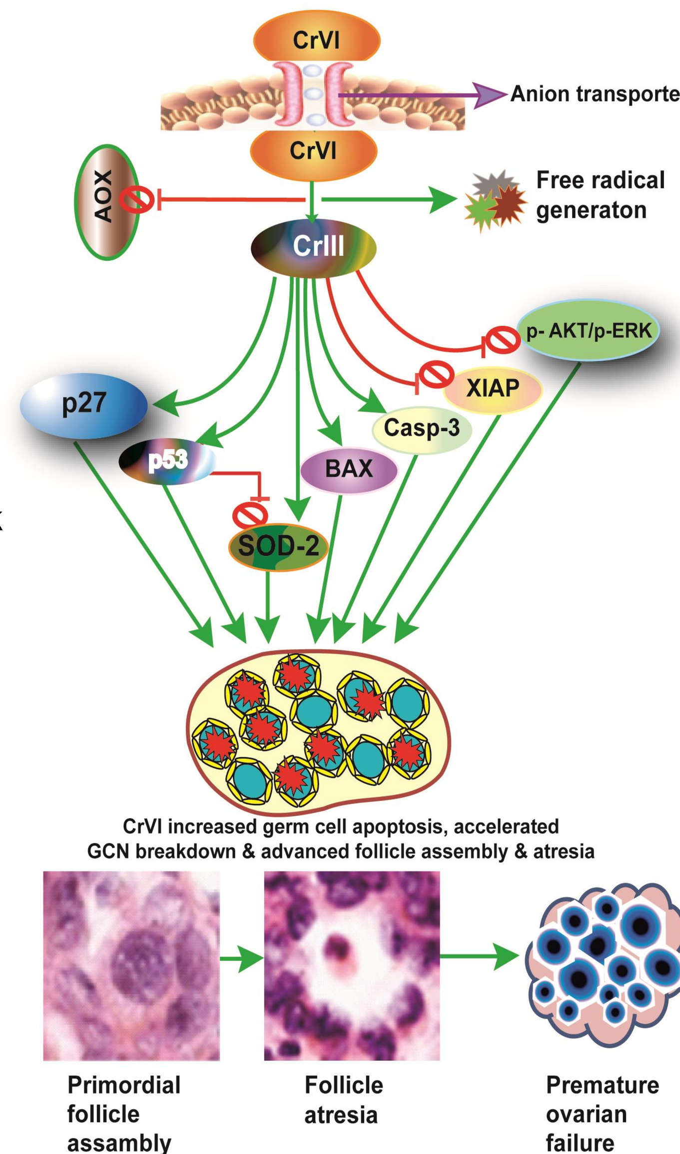
FreshWaterSystems.com 877.335.3339

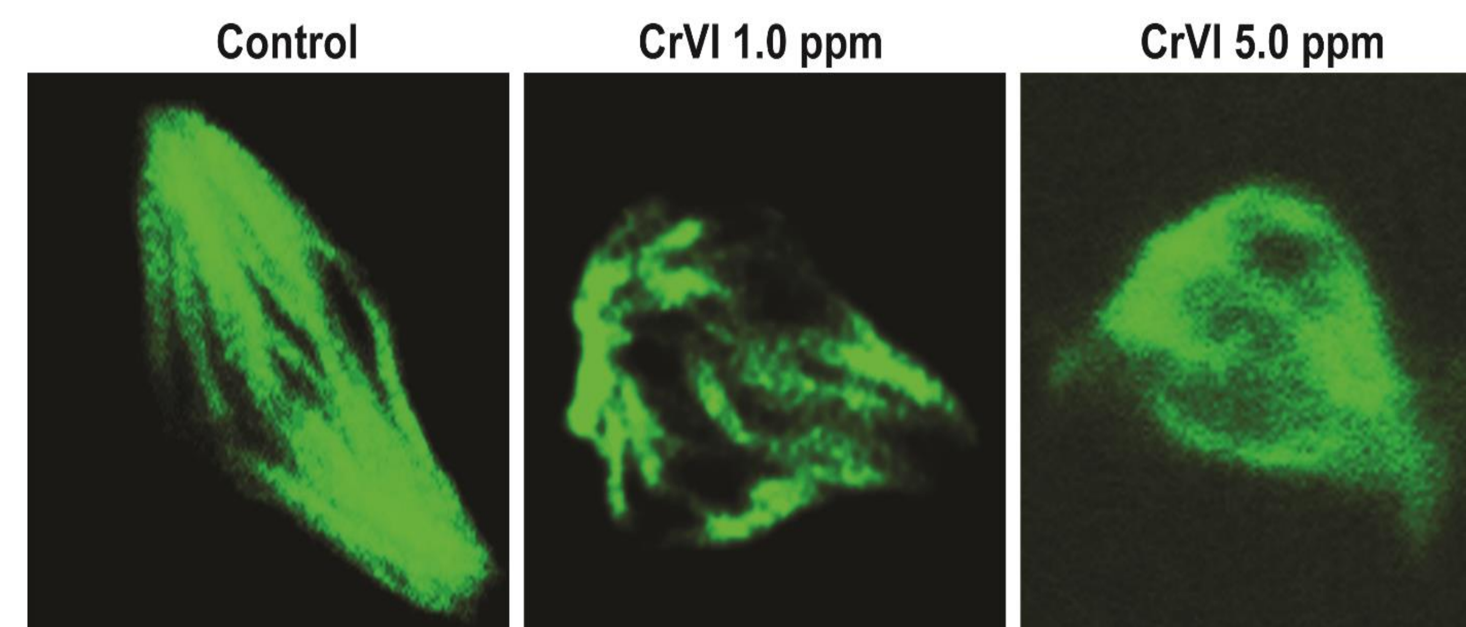
Groundwater Contamination with Chromium-6 in Hinkley, California

THIS PROPERTY AQUIFERS AND DRINKING WATER ARE POISONED

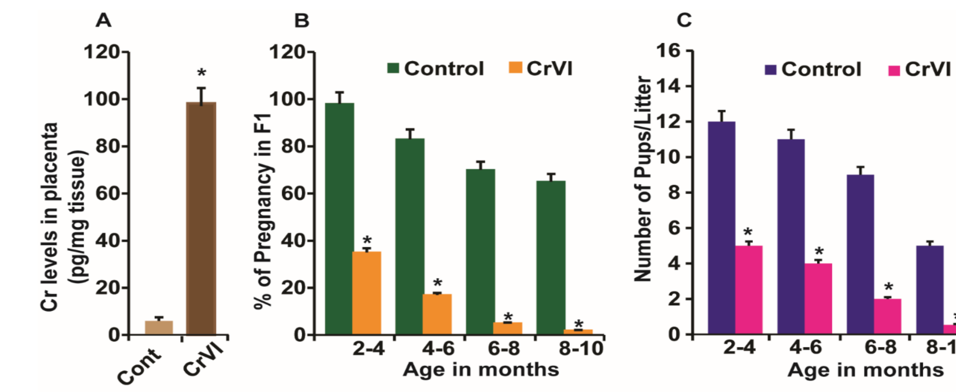


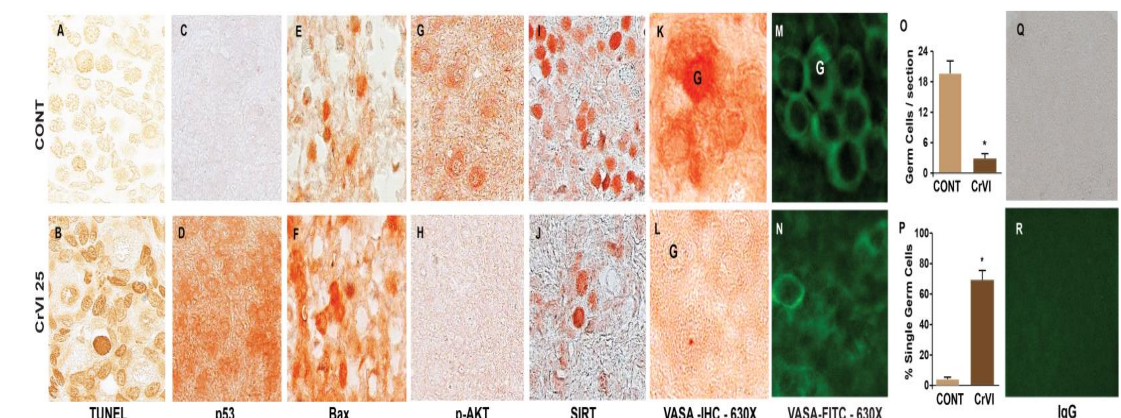






Exposure to Cr(VI) through drinking water causes defective microtubule assembly, disorganization of chromosomes, leading to increased chances of aneuploidy or defective meiotic cell division. Green: Microtubules; Liga Wuri & Banu SK, 2020 (unpublished data).





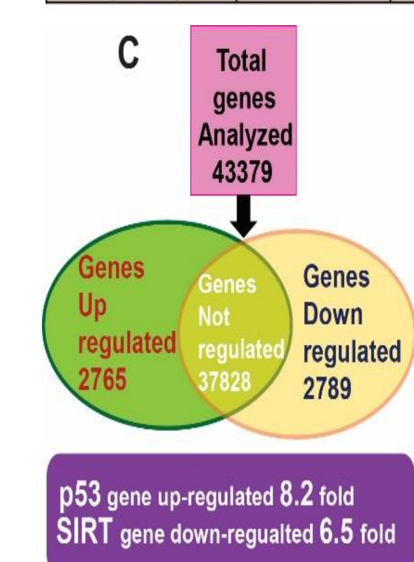
Pregnant F0 mother rats were given regular drinking water (n=5) or exposed to CrVI (potassium dichromate, 25 mg/L) (n=5) through drinking water during gestational days (GD) 8.5 – 12.5. The developing F1 fetuses were exposed to the respective treatment in utero via placental transfer. On PND-1 the female F1 pups (n=20/group) were euthanized and ovaries collected. Randomly, 10 ovaries were fixed in 4% PFA and processed for TUNEL assay and IHC, 10 ovaries were fixed as whole mount to analyze germ cell nest breakdown, and 20 ovaries were used for RNA extraction for miRNA and/or gene expression analyses. ***(A-B)*** *TUNEL assay was performed using in situ TUNEL assay kit as we reported (3).* ***(B-J)*** *Expression of proapoptotic and apoptotic proteins was performed using IHC and quantified using Image Pro Plus as we reported (3). Note: histograms were not shown due to page limit. Expression of VASA-positive germ cells by IHC* ***(K-L)*** *and by whole mounts and confocal microscopy* ***(M-N).*** *Germ cell nest break down was measured by z-stack imaging as described by our collaborator (39). Number of VASA-positive germ cells/section was counted in 32 random fields from 8 ovaries (4 fields/ovary). Percentage of single oocytes was counted in each section and expressed as mean ± SEM* ***(O-P).*** *Data were analyzed by ANOVA. * P<0.05, Control vs. CrVI. Internal control IgG (Q-R).*

CONTROL | CrVI | CONTROL | CrVI
Xpnpep2 | Col3 | Overlay | Xpnpep2 | Col4 | Overlay
Colocalization coefficient

Colocalization of Xpnpep2, a Premature Ovarian Failure marker candidate in the human, and it's Substrates Collagen-3 and Collagen-4 proteins in the Embryonic (day 17.5) ovaries

A	F1 Pups PND-21	Cr in blood (pg/ml)	Cr in the ovary (pg/mg tissue)
	Control (n=10)	2.5 ± 0.2	2.9 ± 0.3
	CrVI (n=10)	13.5 ± 1.0*	12.5 ± 1.3*

B	F1 Pups PND-21	No. of primordial follicles	No. of primary follicles	No. of secondary follicles	No. of atretic follicles
	Control (n=10)	501 ± 25	61 ± 3.8	31 ± 3.8	34 ± 3.0
	CrVI (n=10)	241 ± 11*	40 ± 2.5*	6.1 ± 0.5*	99 ± 7.0*



Pregnant F0 mother rats were given drinking water (n=5) or exposed to (potassium dichromate, 5.0 mg/L) (n=5) through drinking water during gestational days (GD) 8.5 – 12.5. The developing F1 fetuses were exposed to the respective treatment in utero via placental transfer. On PND-21 the female F1 pups (n=10/group) were euthanized, blood and the ovaries collected. Ten ovaries were fixed in 4% PFA, serially cut at 5μm, and every 10th section was examined using H&E. ***(A)*** *Ten ovaries were used for Cr estimation as we described [3,8].* ***(B)*** *The healthy and atretic follicles were counted as we reported [3,8]. Data were analyzed by ANOVA. * P<0.05, Control Vs. CrVI.* ***(C)*** *Remaining 20 ovaries were used for RNA extraction and processed for microarray. Gene expression profiling was performed using Rat Gene Expression Microarray (G2519F-014879), a glass slide formatted with four high-definition 44K arrays and DNA Microarray Scanner, (Agilent Technologies). The protocol provided by "The institute for genomic research" was followed. Data were analyzed using 'R' Statistical software and SAS. * P<0.05, Control Vs. CrVI.* ***Note:*** *Our data indicate that each F0 mother delivered 5 F1 female pups; therefore, the estimated sex ratio is five female F1 pups / F0 mother.*

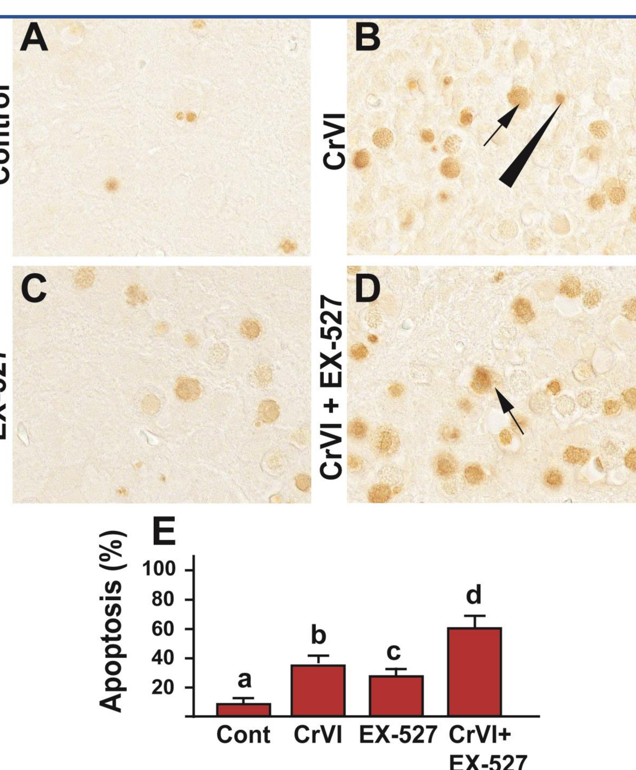
Conclusion: Exposure to hexavalent Cr(VI) through drinking water causes premature ovarian failure by causing germ cell death, disrupting cell survival, activating p53, and inhibiting Sirtuin-1. Cr(VI) disrupts chromosome segregation during oocyte meiotic division. Cr(VI) alters extracellular matrix, and disrupts premature ovarian failure markers. All these molecular changes lead to premature ovarian failure, premature abortions, and infertility in females.

Acknowledgement: Texas A&M Triads for Transformation , A President's Excellence Funds Initiative & NIEHS.

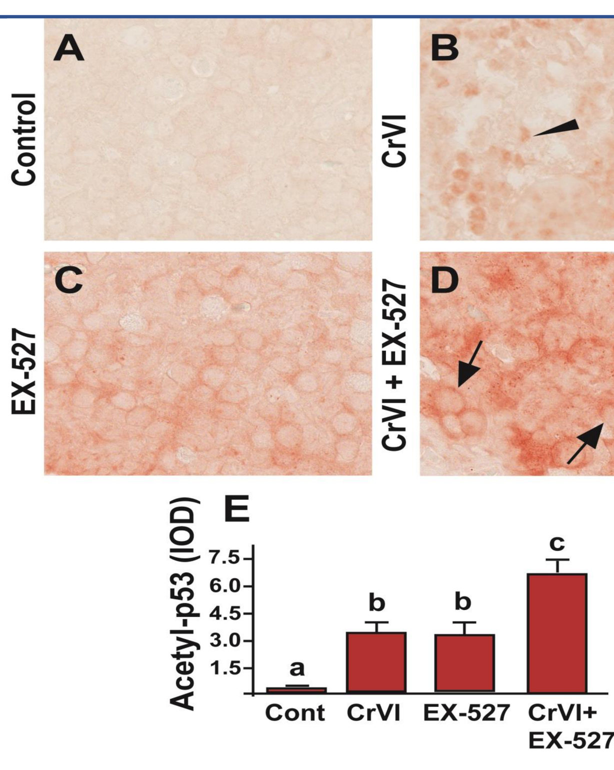
Grants in progress: The data collected using the T3 fund are developed into NIH grants.

CR(VI) INDUCES ACTIVATION OF GERM CELL DEATH.

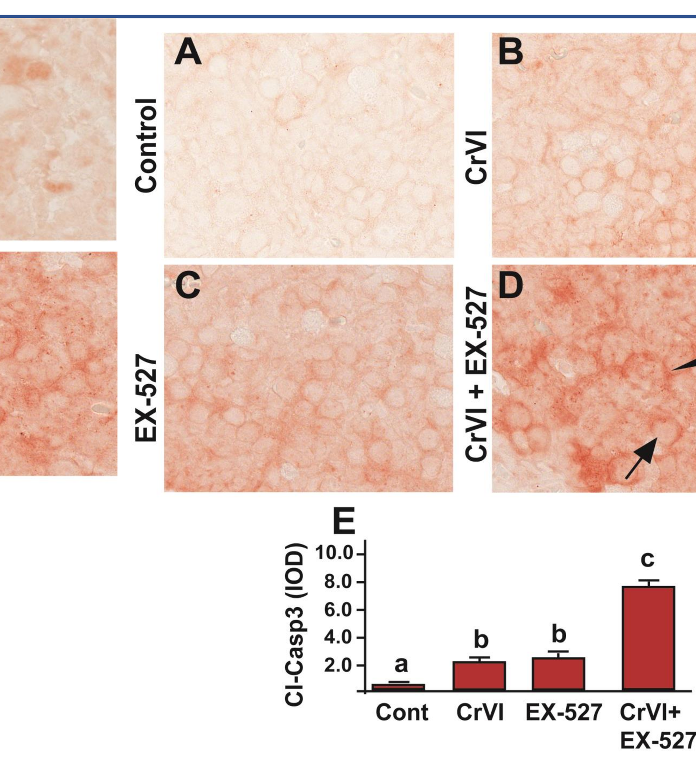
Cr(VI) increases germ cell death; EX-527 accelerates the effects of Cr(VI).



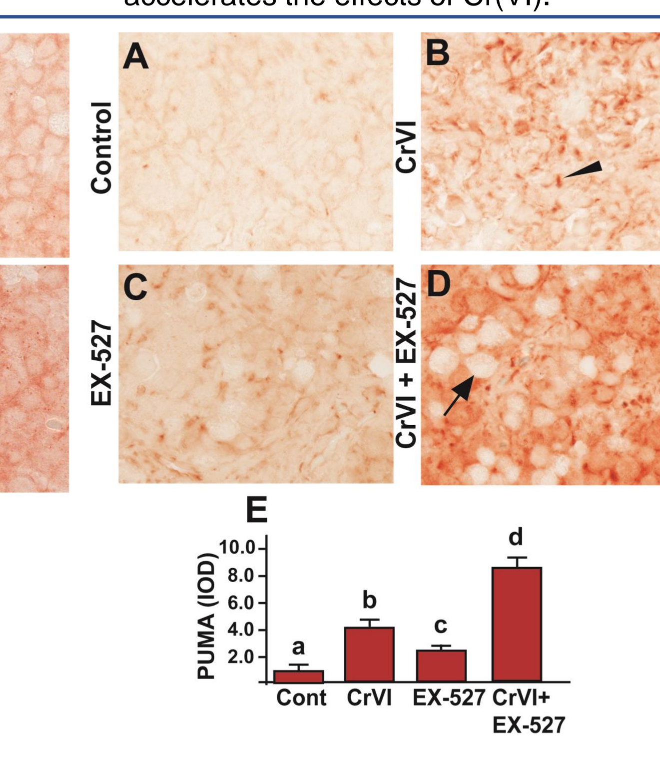
Cr(VI) increases p53 expression; EX-527 accelerates the effects of Cr(VI).



Cr(VI) increases cleaved-casp-3 expression; EX-527 accelerates the effects of Cr(VI).



Cr(VI) increases p53-upregulated modulator of apoptosis (PUMA); EX-527 accelerates the effects of Cr(VI).



CR(VI) INDUCES INHIBITION OF GERM CELL SURVIVAL.

Cr(VI) decreases Bcl-2 expression; EX-527 accelerates the effects of Cr(VI).