Take home messages

1. **Toxic registrations not based on state of the art science. They are based on an *economic* model where the question is: "Do the economic benefits outweigh the risks?", not *is it safe?*. Risks are narrowly defined and do not consider neurological, endocrine, immune or epigenetic risks.**

2. **EPA model assumptions are deficient in *DREAMS. (Bait & Switch)*** Porter, et al. 1999. Toxicol. & Indust. Health. 15 (1-2): 133-150

3. **Opportunity for solution =**
   CHANGE IN MARKET SHARE
4. **Safe alternatives available for weed control, e.g. vinegar.**

DREAMS for public awareness

Dosing deficient - pulse doses at low concentrations not considered (ADHD)

Routes restricted - single exposure routes used; need oral, cutaneous, respiratory routes

Endpoints excessive - cancer and mutations used, need immune, endocrine, nervous systems and development

Additions absent - manufacturing contaminants, toxic waste contaminants deliberately added ('reworking') and 'inert ingredients' (organic soaps & surfactants) missing in 'reagent grade' tests

Mixtures missing - little or no testing for commonly occurring mixtures

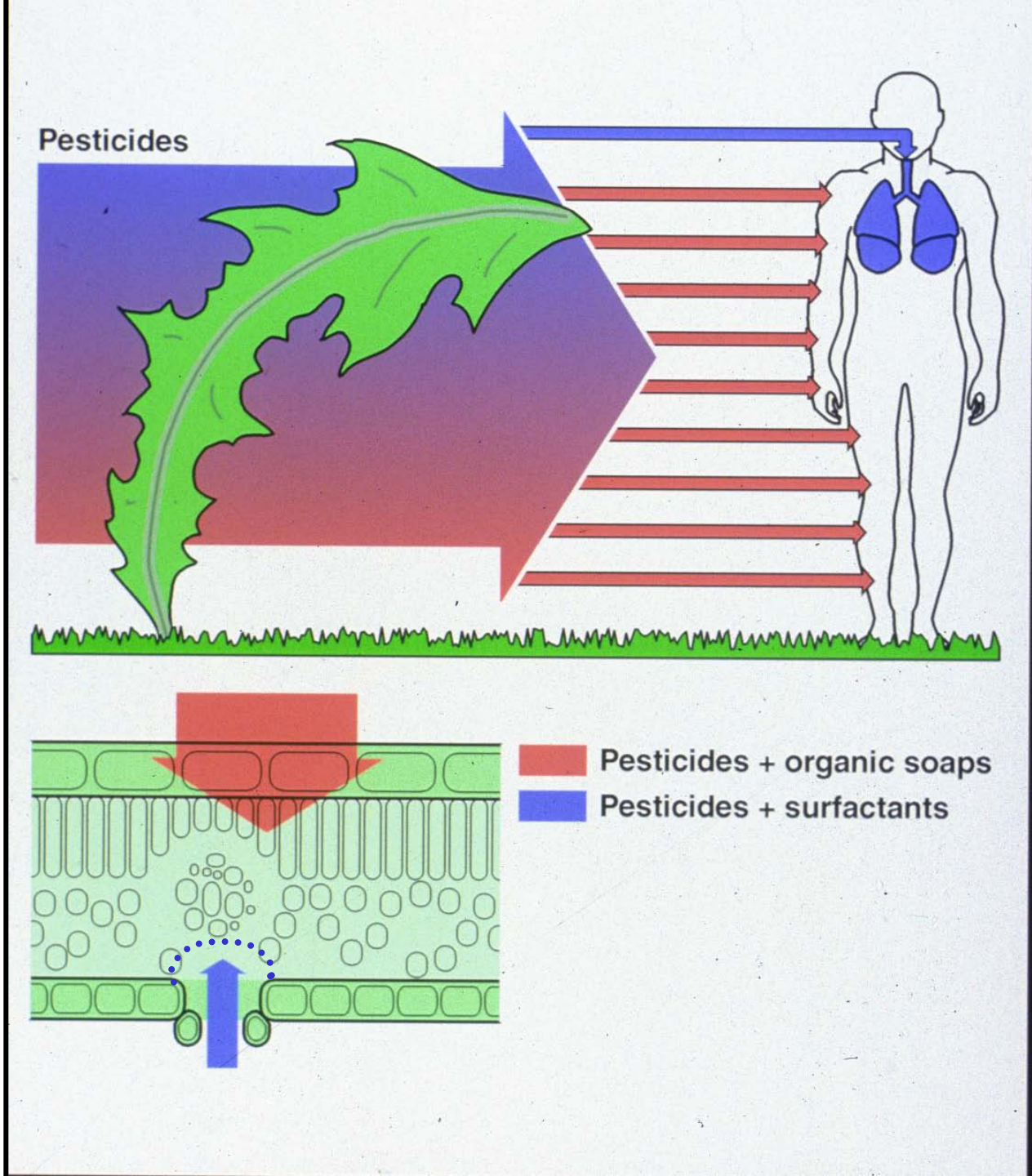
Stresses squelched - nutrition, disease, climate stress not in tests

Why entry is easy -> *other 'inert' ingredients*

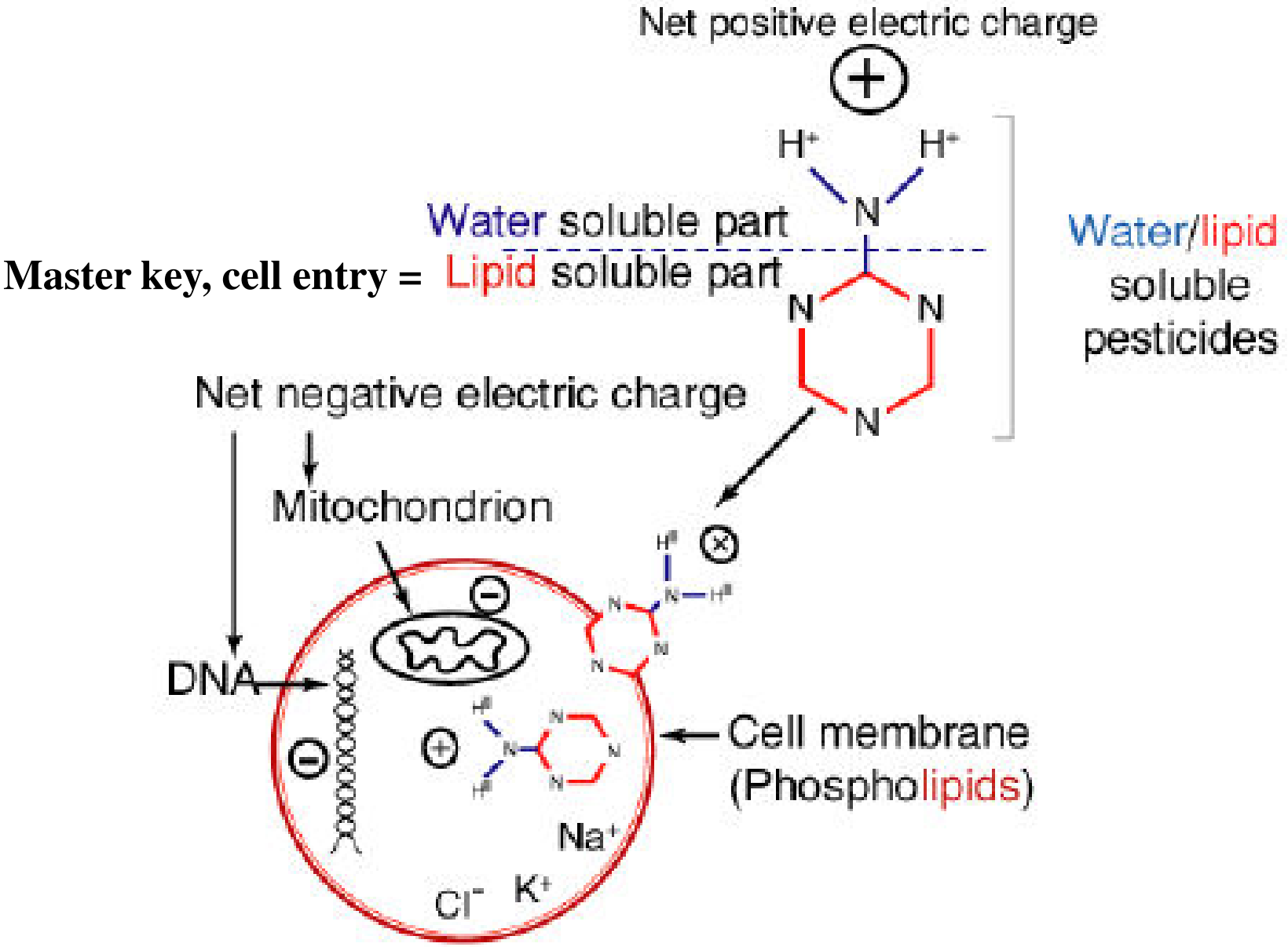
1) non-ionic solvents/ "organic soaps" (= no charges)

&

2) surfactants



Pesticide structure-function
(Why unintended effects are virtually certain)



Master key, cell entry =

RoundUp & defensive enzymes

Many people report experiencing severe digestive problems related to irritation of their gastrointestinal tract after overexposure to RoundUp, limiting the foods their bodies will tolerate to a very few bland foods.

This is believed to be related to the fact that in a 1983 study by Heitanen, Linnainmaa and Vainio, RoundUp's main ingredient, glyphosate, was shown to decrease the hepatic level level of cytochrom P-450, monooxygenase activities, and the intestinal activity of aryl hydrocarbon hydroxylase.

1993. RoundUp mix inhibits defensive enzymes

The inhibition of erythrocyte glutathione conjugate transport by polyethoxylated surfactants has also been reported in a 1993 letter to FEBS from studies done by P. G. Board, part of the Molecular Genetics Group, John Curtin School of Medical Research, Australian National University, Canberra.

Glutathione is a tripeptide which the body produces from the amino acids cysteine, glutamic acid, and glycine. Glutathione is a powerful antioxidant produced in the liver, where it detoxifies harmful compounds so that they can be excreted through the bile. The glutathione released from the liver directly into the bloodstream helps to maintain the integrity of red blood cells and protect white blood cells. Glutathione is also found in the lungs. In the intestinal tract, it is needed for carbohydrate metabolism, and also appears to exert anti-aging effects, aiding in the breakdown of oxidized fats that may contribute to atherosclerosis. Glutathione's role in carbohydrate metabolism is compromised by the effect of RoundUp's surfactant, POEA, on erythrocyte glutathion conjugate transport.

1999. Roundup affects early cell division processes in embryos

Dysfunction in sea urchin early development induced by pesticide treatment.

Marc, J. Mulner-Lorillon, O. Garin S. and Bellé R
Place Georges Teissier Ciudad: Roscoff Provincia: France Código Postal: 29682
belle@sb-roscoff.fr
MARINE TECHNOLOGIES
Marc, J. * Mulner-Lorillon, O. Garin S. and Bellé R. Station Biologique de Roscoff, UPR CNRS 9042, UPMC. Place Georges Teissier, BP74, 29682 Roscoff cedex. Tel : 33 2 98 29 23 46 ; fax : 33 2 98 29 23 06 ;
marc@sb-roscoff.fr

SUMMARY:

Dysfunction in sea urchin early development induced by pesticide treatment. Pollutants from anthropic origin are correlated with human health disorders (Jaga and Brosius, 1999). In order to investigate the chronic effects of pesticides on cell division and early reproduction we analyzed the effect of a commercial glyphosate-containing pesticide during the early phases of sea urchin development. Pollutant molecular targets were searched from comparison between pesticide-induced phenotypes and phenotypes obtained using specific cell cycle effectors (Nigg, 2001). Sea urchin gametes were fertilized in standard laboratory conditions. Embryos were treated with the commercial glyphosate-containing pesticide "Roundup". Dysfunctions in the cell cycle and the early development were monitored by fluorescence microscopy after Hoechst staining of DNA (Delalande et al., 1999). The pesticide, diluted in sea water adjusted to pH 7.5 and added to the embryos 10 minutes post-fertilization induced a highly significant delay in the first cell division of the embryos. M-phase was observed later than in control embryos. The delay was dependent on the pesticide concentration. At a 0.8 % dilution of the commercial pesticide formulation, cytokinesis occurred at 180 min in the treated embryos compared to 120 min in the control foster embryos. Maximal delay in cytokinesis was obtained for a 60 minutes exposure to 0.8 % dilution, applied 10 or 30 minutes post-fertilization. The effect was reversible as judged from development recovery of the embryos after removal of the pesticide. The effect was increased when the pesticide solution was not neutralized. The phenotype induced by the pesticide was comparable to the phenotypes induced by roscovitine, a specific CDK1/ cyclin B inhibitor or by emetine, a protein synthesis inhibitor that impedes CDK1 activation by preventing cyclin B synthesis. Experiments are currently undertaken to determine whether CDK1/ cyclin B activity and/or activation is involved in the pollutant-induced cell cycle effect. The glyphosate-containing pesticide "Roundup" affects cell cycle when present at a concentration of 1/10th of the recommended usage dose for herbicide action. The pesticide concentration that modifies the cell cycle regulators at the molecular level remains to be determined and compared to the residual amount of pollutant found in water. DELALANDE, C., BELLE, R., CORMIER, P. and MULNER-LORILLON, O.(1999). Transient increase of a protein kinase activity identified to CK2 during sea urchin development. Biochim. Biophys. Res. Commun., 266, 425-431. JAGA and BROSIUS (1999). Pesticide exposure: human cancer on the horizon, Rev. Environ. Health, 14, 39-50. NIGG, E.(2001). Mitotic kinases as regulators of cell division and its checkpoints, Nature review, 2, 21-32.

2000. Roundup & steroid hormones

changing reproductive hormones by changing regulatory protein (gene expression)

Environmental Health Perspectives Volume 108, Number 8, August 2000

Roundup Inhibits Steroidogenesis by Disrupting Steroidogenic Acute Regulatory (StAR) Protein Expression

Lance P. Walsh,[1] Chad McCormick,[1] Clyde Martin,[2] and Douglas M. Stocco[1]

[1]Department of Cell Biology and Biochemistry, Texas Tech University Health Sciences Center, Lubbock, Texas, USA
[2]Department of Mathematics, Texas Tech University, Lubbock, Texas, USA

Abstract

Recent reports demonstrate that many currently used pesticides have the capacity to disrupt reproductive function in animals. Although this reproductive dysfunction is typically characterized by alterations in serum steroid hormone levels, disruptions in spermatogenesis, and loss of fertility, the mechanisms involved in pesticide-induced infertility remain unclear. Because testicular Leydig cells play a crucial role in male reproductive function by producing testosterone, we used the mouse MA-10 Leydig tumor cell line to study the molecular events involved in pesticide-induced alterations in steroid hormone biosynthesis. We previously showed that the organochlorine insecticide lindane and the organophosphate insecticide Dimethoate directly inhibit steroidogenesis in Leydig cells by disrupting expression of the steroidogenic acute regulatory (StAR) protein. StAR protein mediates the rate-limiting and acutely regulated step in steroidogenesis, the transfer of cholesterol from the outer to the inner mitochondrial membrane where the cytochrome P450 side chain cleavage (P450scc) enzyme initiates the synthesis of all steroid hormones. In the present study, we screened eight currently used pesticide formulations for their ability to inhibit steroidogenesis, concentrating on their effects on StAR expression in MA-10 cells. In addition, we determined the effects of these compounds on the levels and activities of the P450scc enzyme (which converts cholesterol to pregnenolone) and the 3ß-hydroxysteroid dehydrogenase (3ß-HSD) enzyme (which converts pregnenolone to progesterone). Of the pesticides screened, only the pesticide Roundup inhibited dibutyryl $[(Bu)_2]$cAMP-stimulated progesterone production in MA-10 cells without causing cellular toxicity. Roundup inhibited steroidogenesis by disrupting StAR protein expression, further demonstrating the susceptibility of StAR to environmental pollutants. **Key words**: chemical mixtures, cytochrome P450 side chain cleavage, environmental endocrine disruptor, 3ß-hydroxysteroid dehydrogenase, Leydig cells, Roundup, steroid hormones,

Glyphosate can suppress energy production enzymes 2001

Environmental Research
Volume 85, Issue 3 , March 2001, Pages 226-231

doi:10.1006/enrs.2000.4229 Cite or Link Using DOI

Toxicology Studies

Effect of the Herbicide Glyphosate on Enzymatic Activity in Pregnant Rats and Their Fetuses[*1]

Jorgelina Daruich, Fanny Zirulnik and María Sofía Gimenez

Cátedra de Bioquímica Molecular, Area Química Biológica, Facultad de Química, Bioquímica y Farmacia, Universidad Nacional de San Luis, 5700, San Luis, Argentina

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Abstract

To prevent health risk from environmental chemicals, particularly for progeny, we have studied the effects of the herbicide glyphosate on several enzymes of pregnant rats. Glyphosate is an organo-phosphorated nonselective agrochemical widely used in many countries including Argentina and acts after the sprout in a systemic way. We have studied three cytosolic enzymes: isocitrate dehydrogenase-NADP dependent, glucose-6-phosphate dehydrogenase, and malic dehydrogenase in liver, heart, and brain of pregnant Wistar rats. The treatment was administered during the 21 days of pregnancy, with 1 week as an acclimation period. The results suggest that maternal exposure to agrochemicals during pregnancy induces a variety of functional abnormalities in the specific activity of the enzymes in the studied organs of the pregnant rats and their fetuses.

Author Keywords: glyphosate; dehydrogenases; pregnancy; fetuses.

2005. Roundup's 'other' ingredients double toxic effects at environmentally relevant low doses.

Differential Effects of Glyphosate and Roundup on Human Placental Cells and Aromatase

Sophie Richard, Safa Moslemi, Herbert Sipahutar, Nora Benachour, and Gilles-Eric Seralini

Laboratoire de Biochimie et Biologie Moleculaire, USC-INCRA, Université de Caen, Caen, France

Roundup is a glyphosate-based herbicide used worldwide, including on most genetically modified plants that have been designed to tolerate it. Its residues may thus enter the food chain, and glyphosate is found as a contaminant in rivers. Some agricultural workers using glyphosate have pregnancy problems, but its mechanism of action in mammals is questioned. Here we show that glyphosate is toxic to human placental JEG3 cells within 18 hr with concentrations lower than those found with agricultural use, and this effect increases with concentration and time or in the presence of Roundup adjuvants. Surprisingly, Roundup is always more toxic than its active ingredient. We tested the effects of glyphosate and Roundup at lower nontoxic concentrations on aromatase, the enzyme responsible for estrogen synthesis. The glyphosate-based herbicide disrupts aromatase activity and mRNA levels and interacts with the active site of the purified enzyme, but the effects of glyphosate are facilitated by the Roundup formulation in microsomes or in cell culture. We conclude that endocrine and toxic effects of Roundup, not just glyphosate, can be observed in mammals. We suggest that the presence of Roundup adjuvants enhances glyphosate bioavailability and/or bioaccumulation. *Key words:* adjuvants, aromatase, endocrine disruption, glyphosate, herbicide, human JEG3 cells, placenta, reductase, Roundup, xenobiotic. *Environ Health Perspect* 113:716–720 (2005). doi:10.1289/ehp.7728 available via *http://dx.doi.org/* [Online 25 February 2005]

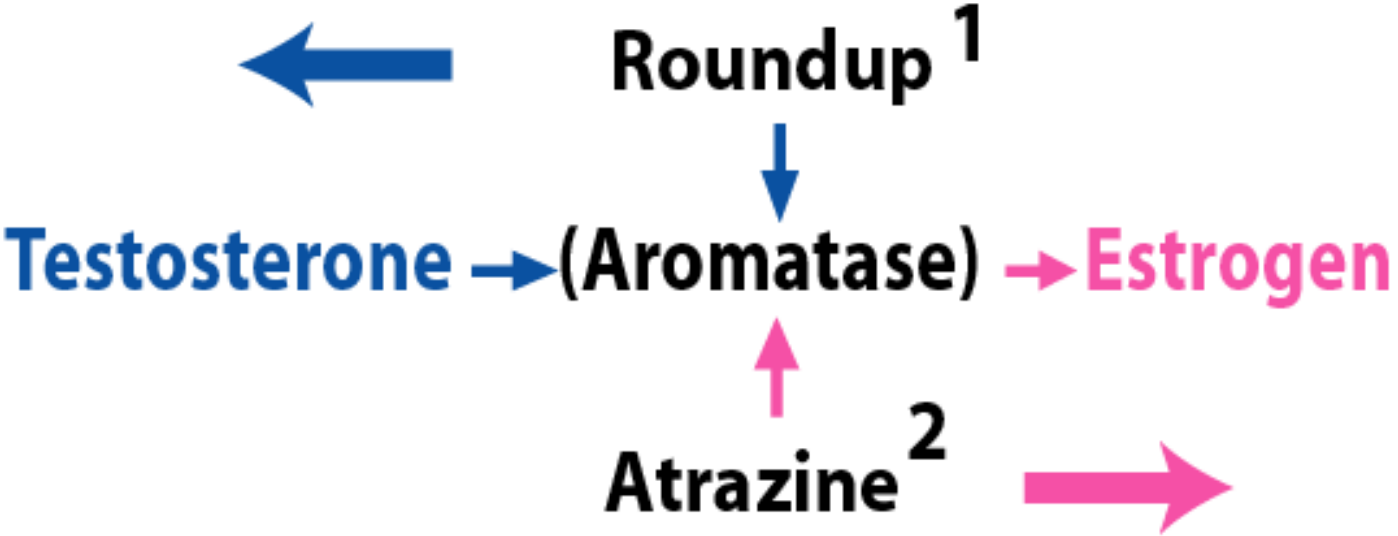
(Saint Quentin Fallavier, France), and the pesticide Roundup (containing 360 g/L acid glyphosate; Monsanto, Anvers, Belgium) was from a commercial source. A 2% solution of Roundup and an equivalent solution of glyphosate were prepared in Eagle's modified minimum essential medium (EMEM; Abcys, Paris, France), and the pH of glyphosate solution was adjusted to the pH of the 2% Roundup solution (~ pH 5.8). Successive dilutions were then obtained with serum-free EMEM. 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) was obtained from Sigma-Aldrich. It was prepared as a 5-mg/mL stock solution in phosphate-buffered saline, filtered through a 0.22-µm filter before use, and diluted to 1 mg/mL in serum-free EMEM. The polyclonal rabbit

"In EPA tests using water from natural sources, the half-life (the time required for a chemical to be reduced to half of its original amount) ranged from 35 to 63 days (1). Persistence in soils is also variable. In Ontario, Canada, glyphosate's half-life in forest soils was 24 days (with detectable residues persisting for 335 days) (2) while a half-life measured in a Finnish study was 249 days (3). Glyphosate was detected in Ohio farming soils 152 days after application of Roundup.(4)"

Glyphosate persistence

(1) U.S. EPA. 1986. Guidance for the reregistration of pesticide products containing glyphosate. Washington, D.C.: Office of Pesticide Programs.
(2) Roy, D.N. et al. 1989. Persistance, movement, and degradation of glyphosate in selected Canadian boreal forest soils. J. Agric. Food Chem. 37:437-440.
(3) Muller, M.M. et al. 1981. Fate of glyphosate and its influence on nitrogen-cycling in two Finnish agricultural soils. Bull. Environm. Contam. Toxicol. 27:724-730.
(4) Edwards, W.M. et al. 1980. A watershed study of glyphosate transport in runoff. J. Environ. Qual. 9(4):661-665.

Common herbicides can alter sex hormones at environmentally relevant concentrations



[1] Richard et al. 2005. Env. Health Persp.

[2] Fan et al. 2007. Env. Health Persp.

Transgenerational Epigenetic Imprinting of the Male Germline by Endocrine Disruptor Exposure during Gonadal Sex Determination

Hung-Shu Chang, Matthew D. Anway, Stephen S. Rekow,
and Michael K. Skinner

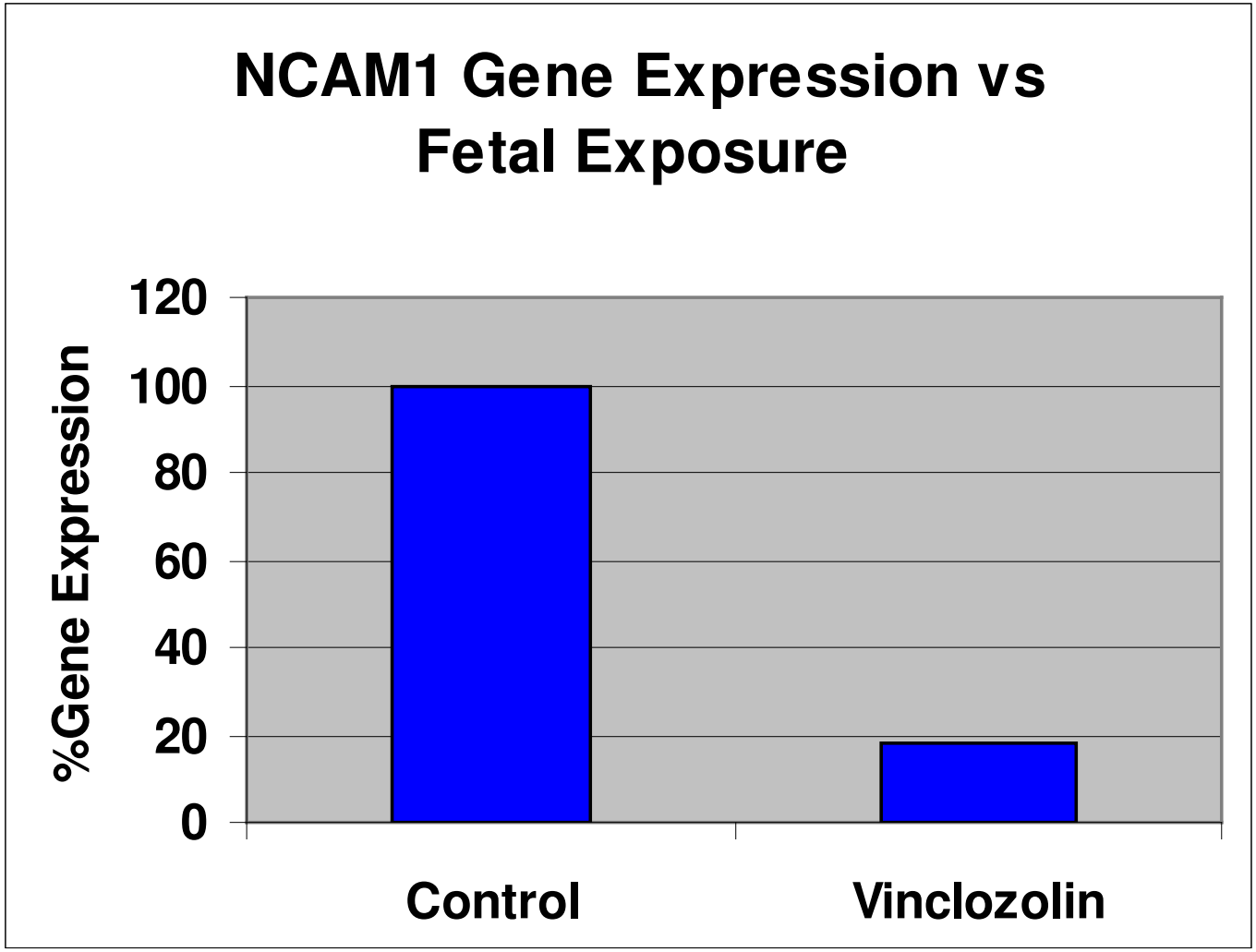
Center for Reproductive Biology, School of Molecular Biosciences,
Washington State University, Pullman Washington
99164-4231

Endocrinology 147(12):5524–5541, 2006

Skinner Found DNA Methylation

- 25 DNA sequences had new methylation sites.
- Each sequence altered neighboring DNA.
- Altered proteins numbered from hundreds to thousands depending upon the fetal organ.
- 1200 alterations in prostate, 800 in brain.

Relative brain expression levels of NCAM1



NCAM1 Gene Related Diseases

- Alzheimers
- Synovial sarcoma
- Schizophrenia
- Mutant-allele-specific amplification (MASA) syndrome
- Neural tube defects
- Various tumors

EPA registration tests for none of these.