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Commentary

Impact of EMS Outreach: Successful Developments in Latin America

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This collection of articles was inspired by the long-standing relationship between the Environmental Mutagen Society and Latin American scientists, and by the program for the 39th Environmental Mutagen Society meeting in Puerto Rico in 2008, which included a symposium featuring ''South of the border'' scientists. This collection, compiled by Graciela Spivak and Ofelia Olivero, both originally from Argentina, highlights scientists who work in or were trained in Latin American countries and in Puerto Rico in a variety of scientific specialties related to DNA repair and cancer susceptibility, genomic organization and stability, genetic diversity, and environmental contaminants. Environ. Mol. Mutagen. 51:763–773, 2010.

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INTRODUCTION

The Environmental Mutagen Society (EMS) has a long history of outreach to scientists and organizations that are concerned with mutagens in the environment in developing countries. In this 40th anniversary year, the EMS celebrates achievements in a number of areas, in which the training, education, and mentoring of scientists and science students worldwide has been a very important part. These activities, which are garnering renewed enthusiasm within the EMS, are pivotal for the generation of ''global scientists.'' The founding members of the EMS (Hollaender, de Serres, Malling, Legator, Freese, and Epstein [Wassom, 1989]) were particularly concerned about the potential genetic impact associated with the proliferation of man-made chemicals. Among those individuals, Hollaender was perhaps the most profoundly influential in promoting scientific communications between the United States and Latin America.

In 1961, nearly half a century ago, Hollaender initiated the Latin American Symposia, a program that promoted scientific exchanges between Latin America and Oak Ridge National Laboratories, of which he was the director [von Borstel, 1987].

Hollaender also organized a number of training courses in a variety of regions including Latin America, in the 1960s–1980s, but these programs ceased after his death in 1986. The courses were reinitiated in 1993, under the title

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TABLE I. Alexander Hollaender Courses

Year	City	Country	Chair(s)
1993	**México City**	**Mexico**	**Patricia Ostrosky-Wegman**
1994	**Buenos Aires**	**Argentina**	**Marta A. Carballo**
1996	Cape Town	South Africa	Wagida Anwar
1997	Cairo	Egypt	Wagida Anwar
1998	**Curitiba**	**Brazil**	**Lucia R. Ribeiro**
1999	Harare	Zimbabwe	Julia Hasler
2000	**Popayán**	**Colombia**	**Luz Stella Hoyos**
2001	Shanghai	China	Jerry Shey
2002	Warsaw	Poland	Antonina Cebulska-Wasilewska
2004	Iasi	Romania	Luminita S. Iancu
2005	Fez	Morocco	Fatma Squali
2006	**Concepción**	**Chile**	**Enrique Zamorano-Ponce**[a]
2007	**Cartagena**	**Colombia**	**Helena Groot**
2008	Kolkata	India	Ashok Giri and William Au
2009	Astana	Kazhakstan	R. Bersimbayev, T. Shalakhmetova, and W. Au

Courses carried out in Latin American countries are shown in bold type.
[a]Zamorano-Ponce, 2008.

''Alexander Hollaender Courses,'' with the meeting organized by Patricia Ostrowski, Michael Shelby, David DeMarini, and Regina Montero in México City. Since then, these courses have taken place nearly every year in countries in which environmental mutagenesis and health issues are major concerns. A complete list of the workshops is shown in Table I; six of them were held in Latin America. Two upcoming courses are also planned for Latin American locations: Viña del Mar, Chile in 2010, and Montevideo, Uruguay in 2011.

Another series of International Conferences on Environmental Mutagens in Human Populations (ICEMHP) was initiated by Drs. W. Au and A. Wagida in 1987 with the purpose of bringing the latest information and technology to less developed areas of the world. The first conference was held in Cairo, Egypt in 1992, and subsequent ones in Prague, Czech Republic (1995), Bangkok and Khao Yai, Thailand (1998), and Florianopolis, Brazil (2003) were just as successful in establishing links and collaborations between scientists. The EMS is one of several organizations that continue to play an important role as sponsor for these conferences.

Without the vision of the founders and their legacy, the efforts and contributions of many scientists in Latin America would have been more difficult to achieve. The creativity and ability to think ''out of the box'' among Latin American scientists has brought many talented individuals to be recognized outside of their native countries, and in occasions to earn prestigious international awards, including the Nobel prize.

The program for the 39th Environmental Mutagen Society meeting in Puerto Rico in 2008 included a symposium showcasing Latin American scientists, in recognition of the unique opportunities presented by the Puerto Rican venue to highlight ''South of the border'' scientists. This session was designed to feature speakers who work in or were trained in Latin American countries in a variety of scientific specialties related to DNA repair and cancer susceptibility, genomic stability and organization, genetic diversity, and environmental contaminants.

We, co-chairs of that symposium, were invited to prepare a manuscript on the Latin American perspective on the role of EMS in improving human health, for this special issue. We decided to let the Latin American scientists speak for themselves: for this collection of papers we have invited outstanding investigators, including some of the participants in the session mentioned earlier, to describe the areas of interest and the research currently being carried out in their laboratories. Their contributions are listed in alphabetical order by country or region of origin; Dr. Zamorano-Ponce's perspective on the history of mutagenesis-focused groups in Latin America concludes the series.

Once again, we and many scientists in Latin America would like to express gratitude for the generous support of our careers by the EMS, and we applaud those EMS members who have held and continue to hold a vision for the successful future of Latin American scientists.

Drs. Olivero and Spivak.

ARGENTINA

A Pesticide or Its Commercial Formulation, Which Is More Deleterious?

Marcelo L. Larramendy and Sonia Soloneski

Living species are unavoidably exposed to pesticides, which represent a significant concern at both ecological and public health levels due to their toxicity. Furthermore, agrochemicals are ubiquitous on the planet because anthropic activities are continuously introducing extensive amounts of these compounds into the environment

(www.epa.gov). In epidemiological as well as in experimental genotoxic and cytotoxic studies, there is an increasing interest in biomonitoring markers to provide both a measurement and an estimation of biologically active/passive exposure to genotoxic pollutants. Despite the beneficial effects associated with the agricultural and household use of agrochemicals, many of them may represent potential hazards due to the contamination of food, water, and air, which can result in severe health problems for humans and ecosystems [WHO, 2006]. Studies in developed countries have demonstrated the annual incidence rates of pesticide intoxication in agricultural workers to be 182 per million full time workers [Calvert et al., 2004] and 7.4 per million among schoolchildren [Alarcon et al., 2005]. Yet, cases of pesticide intoxication may result from various causes in different regions of the world. In emerging countries, where there is insufficient regulation, lack of surveillance systems, less enforcement, lack of training, inadequate access to information systems, poorly maintained or nonexistent personal protective equipment, and larger agriculturally based populations, the incidences are expected to be higher [IFCS, 2003]. The use of pesticides banned in industrialized countries, in particular highly toxic pesticides as classified by WHO [2006], obsolete stockpiles and improper storage techniques may provide unique risks in the developing world [FAO, 2003], where 25% of the global pesticide production is consumed [WHO, 2006]. In Argentina, e.g., available official data revealed that 79% of the intoxications due to pesticides are related with the use of herbicides, followed by insecticides and fungicides (www.msal.gov.ar).

In agriculture, pesticides are generally not used as a single active ingredient but as part of a complex commercial formulation. In addition to the active component, the formulated products contain different solvents and adjuvants, some of which have been reported to induce damage in mammalian cells, among others [Lin and Garry, 2000; Soloneski et al., 2008; González et al., 2007, 2009; Molinari et al., 2009]. Hence, risk assessment must also consider additional toxic effects caused by the excipient/s. Thus, both the workers and non-target organisms are exposed to the simultaneous action of the active ingredient and a variety of other chemical/s contained in the formulated product.

We evaluated comparatively the genotoxic and cytotoxic effects exerted in mammalian cells in vitro by several pure pesticides and their technical formulations commonly used in Argentina. Among them are included the herbicides 2,4-D and 2,4-D DMA®, Dicamba and Banvel®, the fungicides Zineb and Azzurro®, the insecticides Carbofuran and Furadan®, Pirimicarb and Aficida®, and the endectocides Ivermectin and Ivomec®. The sister chromatid exchange, cell-cycle progression, structural chromosomal aberrations, comet, spindle disturbances, micronuclei, mitotic index, MTT, and neutral red assays were used as end-points for geno- and cytotoxicity in several cell systems (human fibroblasts and lymphocytes, and mammalian cell lines). The results clearly demonstrated that the damage induced by the commercial formulations is in general greater than that produced by the pure pesticides, suggesting the presence of deleterious components in the excipients with a toxic additive or/and synergic effect over the pure agrochemicals (Table II). Furthermore, the results highlight the following: (1) a complete knowledge of the toxic effect/s of the active ingredient is not enough in biomonitoring studies; (2) pesticide/s toxic effect/s should be evaluated according to the commercial formulation available in the market; (3) the deleterious effect/s of the excipient/s present within the commercial formulation should not be ignored nor underestimated; and (4) a single bioassay is not enough to characterize the toxicity of an agrochemical under study. These studies were funded by the National University of La Plata (Grants N° 11/N493 and 11/N564) and the National Agency of Scientific and Technological Promotion (Grant BID PICT 2004 N° 26116) from Argentina.

Centrosome Amplification: A New Endpoint?

Ofelia A. Olivero

Patients infected with the human immunodeficiency virus 1 (HIV-1) undergo therapy with antiretroviral nucleoside reverse-transcriptase inhibitors (NRTIs), among which azidothymidine (AZT) is used frequently. AZT becomes incorporated into DNA, where it causes mutations as well as changes in cell cycle and gene expression, and induces S-phase arrest, micronuclei, chromosomal aberrations, sister chromatid exchanges, and telomeric attrition (reviewed in [Olivero, 2007, 2008]). A predicted consequence of these events is genomic instability, which together with clastogenicity may contribute to the carcinogenic potency of AZT. One measure of genotoxicity is centrosomal amplification. Localized by pericentrin antibody [Peloponese et al., 2005], and evidenced by cells containing >2 centrosomes, this endpoint is also an evidence of chromosomal instability [Pihan et al., 1998]. When NRTIs were used in human cells (NHMECs) exposed to two concentrations—a plasma level dose and a dose 10-fold higher—centrosomal amplification was observed in 31.7% of cells exposed to 200 μM AZT for 24 hr and 5.8% of unexposed cells. On the other hand, NHMEC 40 cells, which did not incorporate AZT into DNA, showed centrosomal amplification in 20.0% of AZT-exposed cells and 7.8% centrosomal amplification in unexposed cells. Similar levels of centrosomal amplification were observed in NHMEC strains 05 and 40 exposed to the NRTIs 2′,3′-dideoxyinosine (ddI), 2′,3′-dideoxy-3′-thiacytidine (3TC), and 2′,3′-didehydro-2′−3′-dideoxythy-

TABLE II. Comparison of the Genotoxic and Cytotoxic Effects Induced on Mammalian Cells by Active Ingredients and Commercial Formulations of Several Pesticides[a]

Pesticide type	Name[b]	Cell type	End-points[c]								References
			SCE	SCGE	MNi	SCA	MI	PRI	SD	MTT/NR	
Chlorinated aromatic hydrocarbon acid herbicide	**2,4-D**	CHO-K1	1.6	97.0			0.5	1.0			González et al., 2005
	2,4-D DMA® (62%)	CHO-K1	1.7	70.2			0.7	1.0			
Chlorophenoxy herbicide	**Dicamba**	CHO-K1	1.4	10.7				0.7			González et al., 2007
	Banvel® (57.7%)	CHO-K1	1.6	81.3				0.9			
	Dicamba	Human lymphocytes	1.4				0.2	0.7			González et al., 2006
	Banvel® (57.7%)	Human lymphocytes	1.6				0.01	0.4			
Methyl carbamate insecticide	**Carbofuran**	CHO-K1	1.3		1.5		0.4	0.8		0.6	Soloneski et al., 2008
	Furadan® (47.0%)	CHO-K1	1.2		1.7		0.4	0.8		0.4	
Dimethyl carbamate insecticide	**Pirimicarb**	CHO-K1	1.3			7.4	0.2	0.5			Soloneski and Larramendy, 2009
	Aficida® (50.0%)	CHO-K1	1.3			3.8	0.3	0.5			
Ethylene bis(dithiocarbamate) fungicide	**Zineb**	CHO-K1	2.1	38.5			0.3	0.8	25.1		Soloneski et al., 2002, 2003
	Azzurro® (70.0%)	CHO-K1	1.9	100.0			0.4	0.8	21.9		
	Zineb	HeLa					0.6		7.3		Soloneski et al., 2003
	Azzurro® (70.0%)	HeLa					0.4		9.5		
	Zineb	Human lymphocytes	1.3		7.3	82.0	0.2	0.6			Soloneski et al., 2001
	Azzurro® (70.0%)	Human lymphocytes	1.7		5.7	93.3	0.3	0.6			
	Zineb	Human fibroblasts					0.2		5.3		Soloneski et al., 2003
	Azzurro® (70.0%)	Human fibroblasts					0.1		5.5		
Macrocyclic lactone endectocide	**Ivermectin**	CHO-K1	1.0	1.4			0.2	0.5		0.2	Molinari et al., 2009
	Ivomec® (1.0%)	CHO-K1	1.0	1.3			0.6	0.7		0.2	

[a]The evaluation was performed using end-points for genotoxicity (SCE, sister chromatid exchange frequency; SCGE, single-cell gel electrophoresis; MNi, micronuclei; SCA, structural chromosomal aberrations) and cytotoxicity (MI, mitotic index; PRI, proliferative rate index; SD, spindle disturbances; MTT, 3[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide; NR, neutral red assays).
[b]Names in bold represent active ingredients; the concentrations of the pure active ingredients are shown in parentheses.
[c]Results are expressed as fold-increases over control data.

midine (D4T). This novel mechanism of genotoxicity could be a useful tool to determine the potential aneuploidizing ability of an agent. In that regard, experiments conducted in our lab showed that plasma equivalent doses of AZT and 3TC (lamivudine), administered in vivo to adult pregnant patas monkeys (*Erythrocebus patas*) induced chromosomal losses in the dams. Recent experiments revealed that bone marrow fibroblasts (derived from mesenchymal cells) obtained from infants born to patas dams exposed to human-equivalent doses of either no drug or NRTIs could be stained with the CREST antibody to identify the presence of centromeric proteins, revealing intact chromosomes. The percentage of cells with kinetochore-positive micronuclei observed was 0.15% in cells from the unexposed monkey, and 0.77% and 0.90% in cells from 3TC/AZT-exposed newborns and 1-year-old infants. This finding suggests the existence of an NRTI-induced malfunction in chromosomal segregation occurring in fetal bone marrow as a result of transplacental drug exposure. Therefore, genotoxicity induced by the NRTIs occurs not only after incorporation into DNA, but also by interacting with the mitotic spindle to generate aneuploidy, a novel mechanism of NRTI-induced genotoxicity.

Cockayne Syndrome, UV-Sensitive Syndrome, and Transcription Past Oxidative DNA Lesions

Graciela Spivak

Our research is focused on the repair of DNA modifications due to environmental and endogenous mutagens that can lead to malignancy. We are particularly interested in how human cells process DNA lesions through the global genomic repair (GGR) and transcription-coupled repair (TCR) pathways. Cockayne syndrome (CS) is a human hereditary disease with multiple, severe symptoms that include growth failure, microcephaly with mental retardation, and premature death. UV-sensitive syndrome (UV^SS) is another hereditary disease with mild clinical manifestations and without neurological or developmental abnormalities. CS and UV^SS are genetically heterogeneous, in that they appear in individuals with mutations in *CSB* or in *CSA*, or in a still unidentified gene for UV^SS [Spivak, 2005; Nardo et al., 2009]. Cells from UV^SS and CS exhibit similar hypersensitivity to UV, which results from defective TCR of photoproducts in expressed genes [Spivak et al., 2002]. Interestingly, no predisposition to skin cancer has been reported for either of these syndromes.

The severity of the symptoms in CS patients may be caused by a defect in processing of base damage generated by endogenous reactive oxygen species, with consequent premature cell death in tissues undergoing intense metabolic activity, while UV^SS patients may be proficient in such pathways. In support of this idea, we have shown that CS cells (but not UV^SS cells) are hypersensitive to the oxidants hydrogen peroxide, potassium bromate, and menadione [Spivak and Hanawalt, 2006; Nardo et al., 2009]. Using a host cell reactivation (HCR) assay with UV-irradiated plasmids, we found that expression of the plasmid-encoded *lacZ* gene is reduced in the TCR-deficient CS-B and UV^SS cells. When the plasmids contained the oxidative base lesions thymine glycol or 8-oxo-7,8-dihydroguanine (8-oxoG), CS cells were defective in recovery of expression, whereas UV^SS cells exhibited levels of expression similar to those in wild-type cells [Spivak and Hanawalt, 2006].

Although TCR through nucleotide excision repair has been clearly established for DNA damage induced by ultraviolet light and a number of chemical carcinogens, the evidence for TCR of oxidative base damage (through base excision repair) remains controversial. Our favored model for the mechanism of TCR implicates an arrested RNA polymerase to recruit an excision repair enzyme complex to the transcription-blocking lesion. In support of this model, a strong correlation between transcription arrest by certain lesions in vitro and TCR of those lesions in vivo has been found in most cases analyzed.

Transcription studies from several laboratories, including our own, using DNA substrates containing a single oxidized base positioned at a unique site in the template strand, indicate that RNA polymerases can bypass these lesions, pause transiently at the lesions with eventual completion of full length products, or yield varying proportions of full length and shorter transcripts, depending upon factors such as the promoter, the polymerase, and the nucleotide sequence surrounding the lesions.

To determine whether oxidative lesions may be subject to TCR, we are defining which nucleotide sequence contexts are important, both for the generation of oxidative base damage and with respect to the inhibition of transcription. We carried out in vitro transcription reactions with RNA polymerases (RNAP) and substrates treated with methylene blue plus visible light (MB+VL) to induce a random distribution of 8-oxoG. The ^{32}P-labeled products of the reactions are detected as discrete bands in denaturing polyacrylamide gels. Our preliminary results indicate that RNAP from phage T7, RNAPII purified from rat livers, or human RNAPII from nuclear extracts are arrested at defined sites within the damaged templates. RNA sequencing in future experiments will allow identification of the transcription arrest sites and correlation with sites of oxidation in the templates; addition or depletion of certain factors in the extracts will provide information on their roles in transcriptional bypass of oxidized bases. These studies let the biological system tell us which factors influence the generation of oxidative DNA damage and the inhibition of transcription at particular positions on the DNA template, and they provide an unbiased approach for determining whether the repair of DNA

lesions subject to the base excision repair pathway can be coupled to transcription. This work was supported by a grant CA91456 from the National Cancer Institute, US Department of Health and Human Services.

BRAZIL

Identification of DNA Repair-Related Mutants in *Caulobacter crescentus*

Carlos F. M. Menck

DNA repair and responses to DNA damage have been extensively studied in bacteria, but these studies have been limited to certain groups. The paradigm for these studies has been the gamma proteobacteria *Escherichia coli*, but other groups have also been investigated, such as the firmicutes *Bacillus subtilis* (which has amazing resistance to DNA damage in its dormant spores) and the deinococcus-thermus *Deinococcus radiodurans* (which presents extraordinary resistance to DNA damage agents) [Frenkiel-Krispin and Minsky, 2006]. However, bacterial genome projects revealed that several classical DNA repair genes are absent for certain groups, indicating that different strategies were also developed to prevent cell death after genomic insult. *Caulobacter crescentus* belongs to the alpha proteobacteria group and it has been used as a model for bacterial cell cycle and differentiation studies [Bowers et al., 2008]. The ability to synchronize these cells makes them very attractive for investigations on DNA damage responses in terms of cell cycle, as these studies are not easily performed in other bacteria, including *E. coli*. The in silico analysis of the *C. crescentus* genome indicated that many of the DNA repair pathways are similar to those in *E. coli*; however, certain important genes are absent and others are duplicated, disclosing differences that are relevant to the understanding of how bacteria deal with DNA damage [Martins-Pinheiro et al., 2007]. This was partially confirmed with experiments in vitro, with the discovery of a group of genes involved in a different translesion DNA synthesis pathway (*imuA* and *imuB*, absent in *E. coli*) and the characterization of the SOS regulon in Caulobacter [Galhardo et al., 2005; da Rocha et al., 2008]. Curiously, the analysis of the SOS regulon was performed in a mutant with a disrupted *lexA* gene, which is essential in *E. coli*. Thirty-seven genes were identified as regulated by the LexA protein, including many genes that encode for proteins that act in DNA metabolism, and which were confirmed to be activated under SOS induction, but two genes (that encode the well known helicase DnaB and a hypothetical conserved protein) are intriguingly downregulated during the SOS response.

To have a more complete picture of the genes involved in the DNA damage responses in these bacteria, we have decided to pursue a functional genomics approach. A systematic study was performed by screening a library of ~5,000 Caulobacter mutants, generated by Tn5 transposon insertion, for those affected in DNA damage responses. Two strategies were performed (illustrated in Fig. 1): (1) detection of clones that presented increased numbers of rifampicin colonies, which indicated a spontaneous mutator phenotype, and (2) decreased resistance to genotoxic agents. In the latter strategy, two well-known DNA damaging agents were used, UVB light irradiation or methyl methane sulfonate (MMS) treatment, as they normally affect different cell pathways, expecting that this would disclose a larger range of genes and pathways related to DNA damage responses. Less than 1% of the clones (31) displayed a mutator phenotype, and the genes were identified by DNA sequencing from the Tn5 insertion. Even after highly restrictive conditions, four genes were found to be involved in protecting cells from increased spontaneous mutation frequency in Caulobacter. However, 249 clones (~20%) were found to be sensitive to UVB or MMS, indicating the relevance of the maintenance of genetic stability under DNA damage stress. Many of these clones (29) were sensitive to both treatments. DNA sequencing succeeded for most of these clones (135), and this led to the identification of 102 different genes.

Many of the genes identified using both strategies are orthologs of genes known as related to the processing of DNA damage in other bacteria, confirming that they encode proteins with similar functions. However, many other functional categories were also identified among the selected clones. Membrane transport and cell energetic metabolism were the functions mostly represented; mutations in these genes may affect the oxidative stress in these bacteria and saturate their DNA repair responses. Genes related to gene regulation and signal transduction were also detected in this screening, and they may be intermediates of cell responses to DNA damage. Particularly interesting are kinases, which may provide a clue on how the processing of DNA lesions alerts the cells to control the cell cycle and remove the lesions, a phenomenon basically unknown in bacteria, but well understood in mammalian cells. Many of the genes identified in these clones, however, were not ascribed a function (the encoded proteins are classified in the functional category of hypothetical or hypothetical conserved proteins). Thus, the phenotypes observed in these experiments were the first described for mutations in these genes and may give the first clues to understanding their role in bacteria. Finally, the ability to synchronize Caulobacter, the well established techniques to analyze cell cycle in these bacteria, together with the availability of this collection of mutants related to mutagenesis and DNA damage responses, may provide tools to investigate how bacteria coordinate their division processes under genotoxic stress

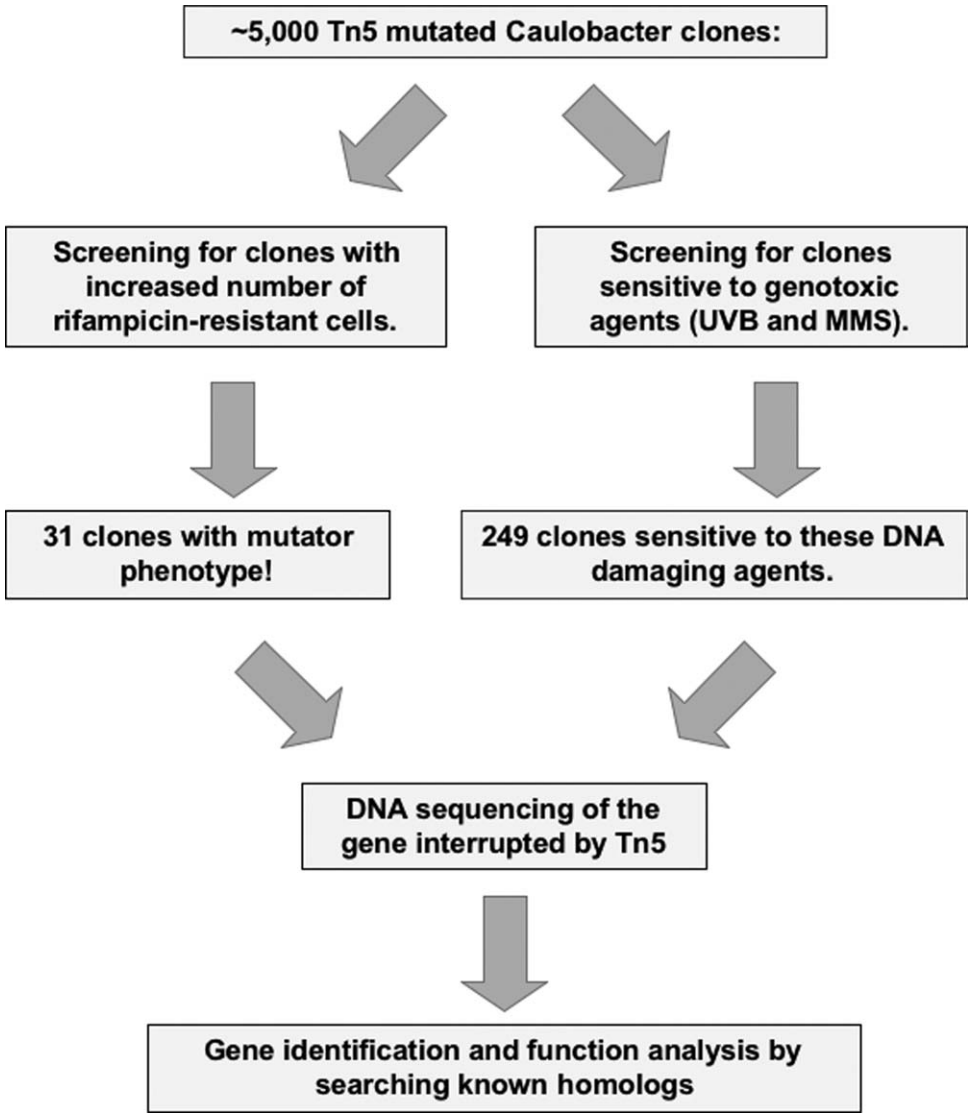

Fig. 1. Strategy for the selection of Caulobacter mutated clones with phenotypes related to spontaneous mutagenesis and DNA damage responses.

conditions, which are situations they normally face in natural environments. This work was supported by FAPESP and CNPq (Brazil).

PUERTO RICO

New Insights into the Role of DNA Repair in Breast Cancer

Jaime Matta

Cancer is the second leading cause of mortality in the US, including Puerto Rico (PR). In women, breast cancer (BC) is the most common invasive cancer worldwide. Data from the Cancer Registry of PR revealed that BC accounted for 31% of all cancers in women in 2003. This represented 1,735 new cases and the highest cancer incidence per organ in women. DNA repair capacity (DRC) is a critical defense system aimed at protecting the integrity and stability of the genome from the harmful effects of cancer-causing agents, including endogenously generated free radicals [Murray and Berg, 2004]. The connection between defects in DNA repair and cancer susceptibility has been known for many years, based on the phenotypes of those rare individuals afflicted with certain rare, genetically determined clinical presentations. Epidemiological studies among the broader population, using functional repair assays in lymphocytes or other accessible cell types, have also demonstrated that DRC is highly variable among individuals, and that a low repair capacity is a significant risk factor for the development of several types of cancers [Mohrenweiser and Jones, 1998; Matta et al., 2003; Murray and Berg, 2004; Ramos et al., 2004; Hu et al., 2007]. Associations between decreased DRC and increased cancer risk among the general population initially became apparent from studies using assays that

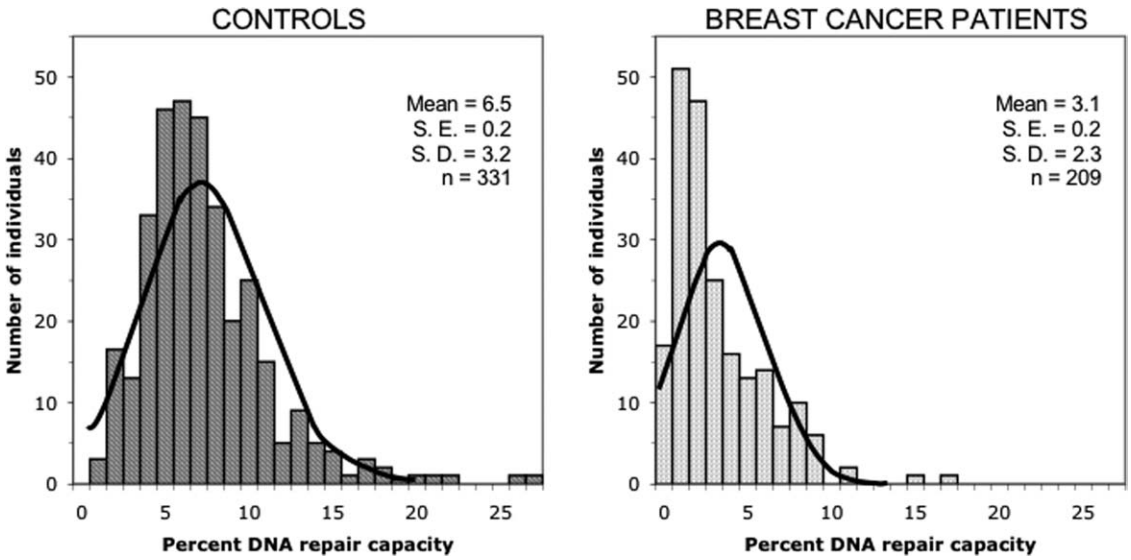

Fig. 2. Percent DRC of women in Puerto Rico with breast cancer (right panel) and controls (left panel). The plasmid expression vector (pCMVluc) was treated in vitro with 700 J/m^2 of UVC and transfected into donors' lymphocytes using DEAE-Dextran transfection reagent (Pharmacia). Luciferase activity was measured 40 hr after transfection.

measure phenotypic repair of DNA damage induced by some external agents, the use of plasmid or viral reactivation assays, or the measurement of levels and splicing patterns of repair gene mRNAs and repair proteins [Murray and Berg, 2004]. The main objectives of this study were to (1) compare the DRC in women with and without breast carcinoma (BC) in PR, and (2) obtain epidemiological data from participants to determine risk factors for breast carcinoma.

This study utilized an incident cases case–control design; it began with recently diagnosed, histopathologically confirmed breast carcinoma cases that had not been treated with radiotherapy and/or chemotherapy. DRC was measured in lymphocytes using the host reactivation assay with a luciferase reporter gene; activity was measured using a luminometer [Matta et al., 2003; Wei et al., 2003]. The percent DRC was calculated based on luminescence counts as the percentage of residual luciferase gene expression (percentage luciferase activity) after repair of damaged plasmid DNA compared with luminescence obtained with undamaged plasmid DNA (100%). The results are illustrated in Figure 2.

Breast cancer cases ($n = 209$) had, on average, 53% less DRC than controls ($n = 331$) ($P < 0.001$, Mann–Whitney U-test). After adjusting for all confounders simultaneously, DRC was a strong predictor for BC. For every unit of decrease in DRC relative to the mean, there was a 64% higher likelihood of having BC. The DRC levels were grouped as high, medium and low. The deleterious effect of low DRC increased from 2.39 (medium vs. high) to 17.2 (low vs. high) more likelihood to have cancer ($P < 0.01$) (data not shown).

These findings support the hypothesis that a low DRC is a risk factor for BC in the Puerto Rican women studied. All pathological types of BC studied were associated with a lower DRC when compared to controls. DRC for all tumor size categories were significantly lower than DRC of controls ($P < 0.05$), but no significant differences in DRC were found between the tumor size groups. In terms of breast cancer grade, contrary to what was initially hypothesized, a lower tumor grade was associated with slightly higher DRC reduction. This may be explained by survival bias.

Age was an important predictor for BC. In average, we found a 3.0% increase in the probability of having BC for every year of age (OR = 1.03, 95% CI: 1.02, 1.05), after adjusting for DRC and other confounders simultaneously. Other predictors significantly associated with BC ($P < 0.05$) were menopause that was a risk factor, endometriosis and vitamin consumption that were protective factors. The statistical significance for some of these variables was lost after multiple adjustments, probably due to the relatively small sample size we currently have for some of these variables.

DRC is an independent and causally related risk factor for BC because it is biologically plausible and shows a strong association and a dose–response relationship after adjusting for all confounders simultaneously. More studies should be conducted to better understand the roles of DRC in the development of BC, while this type of test could be a useful marker in predicting BC risk in the population studied.

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URUGUAY

Linking Genetic Damage to Gene Expression

Gustavo A. Folle

Human chromosomes are composed of alternate Giemsa light (G-light) and dark (G-dark) bands, which exhibit dissimilar DNA base content, gene richness, chromatin structure, replication timing, transcriptional activity and interspersed repeated sequences. G-light bands (R-bands) are G-C-rich, early-replicating, comprise mainly active housekeeping genes and harbor short interspersed repeated sequences (SINEs). Conversely, G-dark bands (or G-bands) are A-T rich, late replicating, encode tissue-specific genes and contain long interspersed repeated sequences or LINEs [Holmquist, 1992].

It has been often claimed that active euchromatic G-light bands are more damage-prone than G-dark bands, even for clastogens with different mechanisms of action such as ionizing radiation (IR), chemical compounds and endonucleases [Folle et al., 1998].

Histone postranslational modifications such as H4 hyperacetylation ($H4^{+a}$) may critically influence gene expression. Transcriptionally active chromatin regularly shows $H4^{+a}$, while constitutive (C-bands) and facultative heterochromatin (inactive X-chromosome, G-bands) are normally underacetylated. Immunodetection of $H4^{+a}$ regions in metaphase chromosomes using fluorochrome-tagged specific antibodies provides a cytogenetic marker for gene expression [Jeppesen, 1997]. As expected, $H4^{+a}$ regions map preferentially to R- and T-bands (T banding identifies the most gene-rich and G-C-rich bands of the human genome, mapping to telomeres [Holmquist, 1992]). Some years ago, we described the colocalization of $H4^{+a}$ regions with breakpoint clusters induced by IR (neutrons, gamma rays) and endonucleases (DNaseI, *Alu*I, and *Bam*HI), thus revealing a marked sensitivity of transcriptionally active domains to genetic damage in Chinese hamster ovary (CHO) chromosomes [Martinez-Lopez et al., 2001].

The extensive analysis of the Human Transcriptome Map (HTM) has allowed the detection of chromosome regions of increased gene expression (RIDGES) and the presence of weakly transcribed domains (antiRIDGEs) [Caron et al., 2001; Gierman et al., 2007].

We have mapped $H4^{+a}$ chromatin regions according to Jeppesen [Jeppesen, 1997], together with RIDGEs, anti-RIDGEs, and transcriptome maps [Gierman et al., 2007] onto human chromosome idiograms (850 bands). In addition, we positioned data on IR breakpoint clusters following Barrios et al. [1989]. We investigated putative colocalization patterns among active chromatin, gene expression levels, and radiation damage.

As expected, nearly all RIDGEs mapped to $H4^{+a}$ chromatin. A total of 69 breakpoint clusters were aligned along G-light or G-dark bands on chromosome idiograms. We were able to determine that the majority of RIDGEs (>60%) colocalized with IR breakpoint clusters. Conversely, only one-third of antiRIDGEs matched radiation clusters exhibiting a lesser amount of breakpoints per cluster when compared to RIDGEs. Moreover, 80% of all radiation breakpoints (55/69) colocalized with $H4^{+a}$ regions.

These findings suggest a link between gene expression and sensitivity to IR damage, giving further support to previous results obtained in CHO cells. However, in human chromosomes not only $H4^{+a}$ regions seem to be more susceptible to genetic damage but also the highly expressed regions in the genome (RIDGEs).

We are presently mapping up- and downregulated tumor genes to disclose potential links with the above-mentioned $H4^{+a}$/RIDGEs/IR clusters in human chromosomes. Finally, since gene-rich and highly expressed chromosome regions tend to reside in the inner portion of nuclei, whereas gene-poor and inactive ones mostly dwell at the nuclear periphery, we also are attempting to analyze the impact of nuclear architecture in cluster generation.

We are greatly indebted to EMS, which has generously provided financial aid for the participation of Uruguayan researchers in two Alexander Hollaender (AH) Courses and the EMS Meeting in Puerto Rico 2008. Moreover, we also wish to thank the encouraging support of the AH Committee to our proposal to hold an A. Hollaender course in Montevideo in 2011.

CONCLUSION: CHILE

Balanced Influence of the EMS in the Development of Mutagenesis Research in Latin America

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The history of Genetic Toxicology is rather recent, with only 72 years since Muller discovered the mutagenic effects of ionizing radiation in Drosophila [Muller, 1927], and it is even shorter in Latin America where the first publications in this area appeared in the late 70s [Takahashi, 1976] and early 80s [Dulout et al., 1980; Ostrosky-Wegman et al., 1984]. Brazil, México, and Argentina were the first countries in which mutagenesis interest groups were organized; these groups played a decisive role in the development of the field in those countries and in others. However, in the 21st century, it is evident that there are vast differences in the scientific area of muta-

genesis between the Latin American countries, due to differences in the percentage of the gross national income (GNI) that each country devotes for scientific and technological advancement. For example, Brazil invests 10-fold more of its GNI for scientific research than Colombia, Peru and Ecuador. The countries that invest the most in the region are Brazil, Argentina, Chile, Cuba, and Costa Rica, while in the remaining countries the amounts are marginal [Larrea, 2006].

On June 9, 1980, a visionary group of Latin American scientists met in Puebla, México, and organized the Latin American Association of Environmental Mutagenesis, Carcinogenesis, and Teratogenesis (ALAMCTA), to represent scientists involved in research on genetic damage induced by environmental chemical and physical agents, on its mechanisms, its effects as well as its prevention, and to promote the application of that knowledge in genotoxicity assays of natural, pharmaceutical, and agricultural products. Since then the advances experienced in some areas have been significant. Today, we can verify the existence of established groups in some countries, and the seeds of groups in others. ALAMCTA today involves scientific societies that promote the advance of mutagenesis studies in Brazil, Mexico, Argentina, Colombia, Bolivia, Paraguay, and Chile. The Environmental Mutagen Society was instrumental in this development, through activities such as the Alexander Hollaender courses, which have inspired dozens of academics, undergraduate and graduate students as well as postdoctoral fellows to orient their studies toward the area of mutagenesis. This has empowered the local scientists and has given them identity and a better sense of the regional realities that merit scientific examination.

ALAMCTA wishes to extend to the EMS Executive Directors and to its members its most heartfelt salutation on this special anniversary, and its appreciation for the constant assistance that has been provided. We express our wish to continue the North-South collaborations that are crucial for the objective of giving continuity to the development achieved thus far, sharing our strengths, increasing the numbers and improving the qualifications of our professional experts, while maintaining our respective national identities.

REFERENCES

Alarcon WA, Calvert GM, Blondell JM, Mehler LN, Sievert J, Propeck M, Tibbetts DS, Becker A, Lackovic M, Soileau SB, Das R, Beckman J, Male DP, Thomsen CL, Stanbury M. 2005. Acute illnesses associated with pesticide exposure at schools. JAMA 294:455–465.

Barrios L, Miro R, Caballin MR, Fuster C, Guedea F, Subias A, Egozcue J. 1989. Cytogenetic effects of radiotherapy. Breakpoint distribution in induced chromosome aberrations. Cancer Genet Cytogenet 41:61–70.

Bowers LM, Shapland EB, Ryan KR. 2008. Who's in charge here? Regulating cell cycle regulators. Curr Opin Microbiol 11:547–552.

Calvert GM, Plate DK, Das R, Rosales R, Shafey O, Thomsen C, Male D, Beckman J, Arvizu E, Lackovic M. 2004. Acute occupational pesticide-related illness in the US, 1998–1999: Surveillance findings from the SENSOR-pesticides program. Am J Ind Med 45:14–23.

Caron H, van Schaik B, van der Mee M, Baas F, Riggins G, van Sluis P, Hermus MC, van Asperen R, Boon K, Voute PA, et al. 2001. The human transcriptome map: Clustering of highly expressed genes in chromosomal domains. Science 291:1289–1292.

da Rocha RP, Paquola AC, Marques Mdo V, Menck CF, Galhardo RS. 2008. Characterization of the SOS regulon of *Caulobacter crescentus*. J Bacteriol 190:1209–1218.

Dulout FN, Larramendy ML, Olivero OA. 1980. Enhancement by caffeine of the frequency of anaphase-telophase chromatin bridges induced by triethylenemelamine (TEM). Experientia 36:346–347.

FAO. 2003. International Code of Conduct on the Distribution and Use of Pesticides. Rome: Food and Agriculture Organization of the United Nations.

Folle GA, Martinez-Lopez W, Boccardo E, Obe G. 1998. Localization of chromosome breakpoints: Implication of the chromatin structure and nuclear architecture. Mutat Res 404:17–26.

Frenkiel-Krispin D, Minsky A. 2006. Nucleoid organization and the maintenance of DNA integrity in *E. coli, B subtilis* and *D. radiodurans*. J Struct Biol 156:311–319.

Galhardo RS, Rocha RP, Marques MV, Menck CF. 2005. An SOS-regulated operon involved in damage-inducible mutagenesis in Caulobacter crescentus. Nucleic Acids Res 33:2603–2614.

Gierman HJ, Indemans MH, Koster J, Goetze S, Seppen J, Geerts D, van Driel R, Versteeg R. 2007. Domain-wide regulation of gene expression in the human genome. Genome Res 17:1286–1295.

González M, Soloneski S, Reigosa MA, Larramendy ML. 2005. Effect of the herbicide 2,4-dichlorophenoxyacetic acid (2,4-D) and its derivative 2,4-D dichlorophenoxyacetic acid dimethylamine salt (2,4-D DMA). I. Genotoxic evaluation on Chinese hamster ovary (CHO) cells. Toxicol In Vitro 19:289–297.

González NV, Soloneski S, Larramendy ML. 2006. Genotoxicity analysis of the phenoxy herbicide dicamba in mammalian cells in vitro. Toxicol In Vitro 20:1481–1487.

González NV, Soloneski S, Larramendy ML. 2007. The chlorophenoxy herbicide dicamba and its commercial formulation banvel induce genotoxicity in Chinese hamster ovary cells. Mutat Res 634:60–68.

González NV, Soloneski S, Larramendy M. 2009. Dicamba-induced genotoxicity oh Chinese hamster ovary (CHO) cells is prevented by vitamin E. J Hazard Mater 163:337–343.

Holmquist GP. 1992. Chromosome bands, their chromatin flavors, and their functional features. Am J Hum Genet 51:17–37.

Hu Z, Wang LE, Wei Q. 2007. Molecular epidemiology of DNA repair and cancer susceptibility—A review of population-based studies. In: Wei Q, Lei L, Chen D, editors. DNA Repair, Genetic Instability, and Cancer. Singapore: World Scientific. pp 315–343.

IFCS. 2003. Acutely toxic pesticides: Initial input on extent of problem and guidance for risk management. Fourth session of the Intergovernmental Forum on Chemical Safety. Doc number: IFCS/FORUM-IV/10w.

Jeppesen P. 1997. Histone acetylation: A possible mechanism for the inheritance of cell memory at mitosis. Bioessays 19:67–74.

Larrea C. 2006. Universidad, investigación científica y desarrollo en América Latina y el Ecuador. Universidad y cooperación para el desarrollo. Universidad Complutense de Madrid.

Lin N, Garry VF. 2000. In vitro studies of cellular and molecular developmental toxicity of adjuvants, herbicides, and fungicides commonly used in Red River Valley, Minnesota. J Toxicol Environ Health 60:423–439.

Martinez-Lopez W, Folle GA, Obe G, Jeppesen P. 2001. Chromosome regions enriched in hyperacetylated histone H4 are preferred sites for endonuclease- and radiation-induced breakpoints. Chromosome Res 9:69–75.

Martins-Pinheiro M, Marques RC, Menck CF. 2007. Genome analysis of DNA repair genes in the alpha proteobacterium Caulobacter crescentus. BMC Microbiol 7:17

Matta JL, Villa JL, Ramos JM, Sanchez J, Chompre G, Ruiz A, Grossman L. 2003. DNA repair and nonmelanoma skin cancer in Puerto Rican populations. J Am Acad Dermatol 49:433–9.

Mohrenweiser HW, Jones IM. 1998. Variation in DNA repair is a factor in cancer susceptibility: A paradigm for the promises and perils of individual and population risk estimation? Mutat Res 400:15–24.

Molinari G, Soloneski S, Reigosa MA, Larramendy ML. 2009. In vitro genotoxic and citotoxic effects of ivermectin and its formulation ivomec® on Chinese hamster ovary (CHO_{K1}) cells. J Hazard Mater 165:1074–1082.

Muller HJ. 1927. Artificial transmutation of the gene. Science 66:84–87.

Murray D, Berg A. 2004. Relationship among DNA repair genes, cellular radiosensitivity, and the reponse of tumors and normal tissues to radiotherapy. In: Panasci L, Alaoui-Jamali M, editors. DNA Repair in Cancer Therapy. New York: Humana Press. p 211–256.

Nardo T, Oneda R, Spivak G, Vaz B, Mortier L, Thomas P, Orioli D, Laugel V, Stary A, Hanawalt PC, et al. 2009. A UV-sensitive syndrome patient with a specific CSA mutation reveals separable roles for CSA in response to UV, oxidative DNA damage. Proc Natl Acad Sci USA 106:6209–6214.

Olivero OA. 2007. Mechanisms of genotoxicity of nucleoside reverse transcriptase inhibitors. Environ Mol Mutagen 48:215–223.

Olivero OA. 2008. Relevance of experimental models for investigation of genotoxicity induced by antiretroviral therapy during human pregnancy. Mutat Res 658:184–190.

Ostrosky-Wegman P, Garcia G, Arellano L, Espinosa JJ, Montero R, Cortinas de Nava C. 1984. Genotoxicity of antiamebic, anthelmintic, and antimycotic drugs in human lymphocytes. Basic Life Sci 29 Pt B:915–25.

Peloponese JM Jr, Haller K, Miyazato A, Jeang KT. 2005. Abnormal centrosome amplification in cells through the targeting of Ran-binding protein-1 by the human T cell leukemia virus type-1 Tax oncoprotein. Proc Natl Acad Sci USA 102:18974–18979.

Pihan GA, Purohit A, Wallace J, Knecht H, Woda B, Quesenberry P, Doxsey SJ. 1998. Centrosome defects and genetic instability in malignant tumors. Cancer Res 58:3974–3985.

Ramos JM, Ruiz A, Colen R, Lopez ID, Grossman L, Matta JL. 2004. DNA repair and breast carcinoma susceptibility in women. Cancer 100:1352–1357.

Soloneski S, Larramendy ML. 2010. Sister chromatid exchanges and chromosomal aberrations in Chinese hamster ovary (CHO-K1) cells treated with the insecticide Pirimicarb. J Hazard Mater 15:410–415.

Soloneski S, González M, Piaggio E, Apezteguía M, Reigosa MA, Larramendy ML. 2001. Effect of dithiocarbamate pesticide zineb and its commercial formulation azzurro. I. Genotoxic evaluation on cultured human lymphocytes exposed in vitro. Mutagen 16:487–493.

Soloneski S, González M, Piaggio E, Reigosa MA, Larramendy ML. 2002. Effect of dithiocarbamate pesticide zineb and its commercial formulation azzurro. III. Genotoxic evaluation on Chinese hamster ovary (CHO) cells. Mutat Res 514:201–212.

Soloneski S, Reigosa MA, Larramendy ML. 2003. Effect of dithiocarbamate pesticide zineb and its commercial formulation azzurro. V. Abnormalities induced in the spindle apparatus of transformed and non-transformed mammalian cell lines. Mutat Res 536:121–129.

Soloneski S, Reigosa MA, Molinari G, González NV, Larramendy ML. 2008. Genotoxic and cytotoxic effects of carbofuran and furadan® on Chinese hamster ovary (CHO_{K1}) cells. Mutat Res 656: 68–73.

Spivak G. 2005. UV-sensitive syndrome. Mutat. Res. 577:162–169.

Spivak G, Hanawalt PC. 2006. Host cell reactivation of plasmids containing oxidative DNA lesions is defective in Cockayne syndrome but normal in UV-sensitive syndrome fibroblasts. DNA Repair (Amst) 5:13–22.

Spivak G, Itoh T, Matsunaga T, Nikaido O, Hanawalt P, Yamaizumi M. 2002. Ultraviolet-sensitive syndrome cells are defective in transcription-coupled repair of cyclobutane pyrimidine dimers. DNA Repair (Amst) 1:629–43.

Takahashi CS. 1976. Cytogenetical studies on the effects of high natural radiation levels in Tityus bahiensis (Scorpiones, Buthidae) from Morro do Ferro, Brazil. Radiat Res 67:371–81.

von Borstel R. 1987. Alexander Hollaender, in memorian. Mutagen 2:149–150.

Wassom J. 1989. Origins of genetic toxicology and the Environ Mutagen Society. Environ Mol Mutagen 14 (Suppl 16):1–6.

Wei Q, Lee JE, Gershenwald JE, Ross MI, Mansfield PF, Strom SS, Wang LE, Guo Z, Qiao Y, Amos CI, et al. 2003. Repair of UV light-induced DNA damage and risk of cutaneous malignant melanoma. J Natl Cancer Inst 95:308–15.

WHO. 2006. Recommended Classification of Pesticides by Hazard and Guidelines to the Classification. Geneva:World Health Organization. 1–200 p.