

TOXICOLOGICAL HIGHLIGHT

Intercellular Communication, Homeostasis, and Toxicology

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The article highlighted in this issue is “Inhibition of Gap Junctional Intercellular Communication by Perfluorinated Compounds in Rat Liver and Dolphin Kidney Epithelial Cell Lines *in Vitro* and Sprague-Dawley Rats *in Vivo*” by W. Hu, P. D. Jones, B. L. Upham, J. E. Trosko, C. Lau, and J. P. Giesy (pp. 429–436).

The evolution of multicellular organisms from unicellular ones required that cells develop methods to “communicate” with neighboring and distant cells within the organism (Gerhart, 1999). This intercellular communication was critical for the evolution of organs and groups of specialized cells within organs (tissues), as well as the regulation of proliferation, apoptosis, and differentiation of specialized cells (tissue homeostasis).

Over the course of evolution, several forms of intercellular communication have appeared (Gerhart, 1999). Cells communicate by releasing soluble factors (e.g., hormones, growth factors, lipid mediators, cytokines, nitric oxide, etc.) that activate other cells locally or distantly through receptors or non-receptor mechanisms. Nerve and muscle cells communicate by chemical and electrical synapses. The integrins are a large family of proteins that are expressed on cell surfaces and are components of hemidesmosomes and focal adhesion plaques. Integrins bind to extracellular matrix molecules secreted by other cells in a highly specific manner, and binding can activate intracellular signal transduction pathways that lead to altered cellular states. Similarly, tight junctions, desmosomes, and adherens junctions do more than connect cells together or block molecular diffusion between them; they also are involved in intracellular signaling. Lastly, one of the most ancient forms of intercellular communication occurs via gap junctions. These junctions consist of aggregated channels that span the plasma membranes of adjacent cells and directly connect their interiors. Gap junctions, unlike other modes of intercellular communication, are present in all animal phyla and provide a direct pathway for the exchange of “molecular information” between cells. The vertebrate gap junction channels have a permeability size limit of approximately 1 kDa, so that amino acids, sugars, nucleotides, most second messengers,

water and other small molecules diffuse between cells through gap junction channels, whereas macromolecules cannot. This cell–cell diffusion is known as gap junctional intercellular communication (GJIC), and it is important for homeostasis, the synchronization of cellular activities, and the regulation of cell proliferation and apoptosis (Ruch, 2000).

These many modes of intercellular communication are involved in all physiological activities of multicellular organisms, and alterations of intercellular communication (increased, decreased, or replacement with a different type) contribute to many, if not all, diseases. Intercellular communication is critical for normal embryogenesis and development, neural activity, gamete production, parturition, endocrine function, immune function, cardiovascular function, and the regulation of cell proliferation, apoptosis, and differentiation (to name just a few). Not surprisingly, defects in intercellular communication can lead to teratogenesis, neuropathy, infertility, diabetes, autoimmune disorders, atherosclerosis, cancer, and other diseases (Trosko *et al.*, 1998).

This central role of intercellular communication in normal physiology and disease necessitates that toxicologists understand if and how toxic agents affect intercellular communication in order to fully understand toxic processes and mechanisms. It is certainly important and appropriate to determine how a toxic agent impinges upon a specific molecule or intracellular process, but one cannot fully understand toxic mechanisms in a multicellular organism without studying intercellular communication. Fortunately, within the past decade, toxicologists have begun to move away from the investigation of single molecules and discrete pathways to study global changes in gene expression, protein activities, and signaling cascades. Advances in genomics, proteomics, and bioinformatics have greatly facilitated this. Still, however, there has been relatively less study of intercellular communication in toxicology except within the areas of neural and endocrine toxicology and tumor promotion/carcinogenesis. Clearly, toxic agents can impinge upon all modes of intercellular communication. So it is likely that investigation of such actions will be very fruitful and lead to a greater understanding of the mechanisms and risk of toxic agents.

The highlighted article by Hu *et al.* (2002) reports the effects of perfluorinated organic compounds on gap junctional inter-

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cellular communication (GJIC) in dolphin kidney and rat liver epithelial cells *in vitro* and rat liver *in vivo*. These agents are widely used in industry and are persistent environmental contaminants; they have been detected in wildlife and humans (Giesy and Kannan, 2001; Renner, 2001). Perfluorinated organics were thought to be nontoxic and metabolically inert, but in fact they have many biological and toxicological actions (Renner, 2001).

Hu *et al.* (2002) characterized the effects of perfluorooctane sulfonic acid (PFOS), perfluorohexane sulfonic acid (PFHS), perfluorobutane sulfonic acid (PFBS), and perfluorooctanoic sulfonamide (PFOSA) on GJIC in WB-F344 rat liver epithelial cells and CDK dolphin kidney epithelial cells *in vitro* and hepatocytes in Sprague-Dawley rat liver *in vivo*. Perfluorinated fatty acids have toxicological effects including the inhibition of GJIC (Upham *et al.*, 1998), but the sulfonated derivatives, which are more prevalent in the environment, have not been as widely studied. The rat liver and dolphin kidney cell lines were chosen because perfluorinated compounds are hepatotoxic and are found in marine mammals (Giesy and Kannan, 2001). PFOS, PFOSA, and PFHS, but not PFBS, decreased GJIC in the cell lines in a dose-dependent, time-dependent, and reversible manner. Based upon the no-observable-effect level (NOEL) and 50% effective concentration (EC₅₀), PFOS and PFOSA were equally potent in the two species of cells and were more potent than PFHS. These data suggested that the carbon chain length was more critical for inhibition than the functional group. GJIC in rat liver hepatocytes *in vivo* was also reduced by the administration of PFOS for 3 or 21 days, with no sex-dependent differences. Hepatocyte gap junction channels *in vivo* are formed by the gap junction proteins, connexin26 and connexin32, whereas those in the liver and kidney epithelial cell lines contain connexin43. This suggests that PFOS could affect GJIC in many tissues, since these connexins are widely expressed. Therefore, the agent might be a risk to human and animal health.

The data indicate that one form of intercellular communication, GJIC, is sensitive to sulfonated derivatives of perfluorinated fatty acids. But does blocked GJIC contribute to the toxic manifestations of perfluorinated fatty acids (e.g., liver necrosis, hepatocarcinogenesis, peroxisome proliferation, and induction of drug metabolizing enzymes; Renner, 2001) observed in rodent bioassays? The study did not answer this question, but it is a difficult one, as are many cause and effect questions in biology and toxicology. Nonetheless, it is likely that the inhibition of hepatic GJIC had additional effects on the organism, e.g., altered glycogen metabolism (Nelles *et al.*, 1996), hormonal response (Leite *et al.*, 2002), and growth control/neoplasia (Temme *et al.*, 1997), that might have contributed to the

toxic outcomes or led to others not evaluated. The paper by Hu *et al.* (2002) is important because it not only has broadened our understanding of the *cellular effects* of sulfonated derivatives of perfluorinated fatty acids, but also because it suggests a likely and often rarely considered mechanism for the *toxicological outcomes* observed in whole animal bioassays.

As noted above, many forms of intercellular communication are involved in homeostasis and normal physiology. It is highly likely that toxicant-induced changes in intercellular communication are frequent and central to toxic manifestations in the whole organism. Because agents that reduce GJIC also likely have other actions at the molecular, cellular, tissue, and organ levels, the next challenge for Hu *et al.*, as well as all other toxicologists, is to integrate these effects into a comprehensive understanding of mechanism. This will improve our ability to quantify risk and determine safe exposure levels for humans and wildlife.

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