aggregates in Eprex did not increase after the formulation change, and was certainly not higher than in other erythropoiesis-stimulating agents[11].

We agree with Schellekens & Jiskoot that it is important to monitor the incidence of immunogenicity in patients treated with biopharmaceuticals. There is also a need for standardized assays to detect antibodies against these therapeutic proteins. As demonstrated by our research, in addition to known factors that can influence the immunogenicity of therapeutic proteins, factors that are less often considered (e.g., container closures) may also have a significant impact by affecting product integrity.

J&J and independent researchers have made an effort to make the research related to the investigations available to the public through the publication of several articles[1,2,4,8–11]. In addition, the data were reviewed by regulatory authorities, including the Therapeutic Goods Administration (Australia), Health Canada and the European Medicines Agency, which have approved the reintroduction of s.c. administration of Eprex, thereby independently confirming the validity of the data demonstrating the safety of the currently marketed formulation of Eprex, including a reduction of EPO antibody-mediated PRCA to the baseline level observed with any erythropoiesis-stimulating agent.

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Schellekens and Jiskoot respond:

In contrast to the assumption of Sharma *et al.*, we scrutinized all the (highly redundant) publications of J&J concerning their explanation of Eprex-associated PRCA. In all of their papers, the only support for a modest adjuvant effect of leachables is a single experiment in mice immunized with ovalbumin. Because this mouse model is not based on breaking B-cell tolerance, this experiment is irrelevant for the induction of antibodies by Eprex. And as confirmed in many models, immune tolerance to soluble self proteins like erythropoietin cannot be broken by an adjuvant.

Sharma *et al.* refer to results of Yano *et al.*[1] for confirmation of the immune stimulant effect of the alkyl phenols present in the leachates. In the Yano *et al.* paper, however, none of the compounds identified as leachates by Sharma *et al.*[2] are studied. Yano *et al.* showed only that this class of compounds has no effect on interleukin 4 and interferon γ production *in vitro* in the concentrations present in Eprex with uncoated rubber stoppers. In higher concentrations, alkyl phenols inhibit immune stimulatory cytokine products—exactly the opposite activity that Sharma *et al.* claim.

Sharma *et al.* agree with us that analyses-based spontaneous reporting should be interpreted with caution, the same caution that the European Medicines Agency has shown by allowing the limited reintroduction of the subcutaneous use of Eprex with chronic renal failure under strict patient surveillance.

Thus, in contrast to the claim of Sharma *et al.*, the European regulatory agencies still need further data to confirm the safety of Eprex. And even if the data were to show the reduction of Eprex-associated PRCA to background levels, this cannot be interpreted as definitive proof that leachates were responsible for the Eprex-associated cases, considering the other changes that have been introduced.

We recently published[3] a detailed analysis of all the theories offered to explain the higher incidence of PRCA after the formulation change of Eprex. Our conclusion is that the formulation change resulted in a slightly less stable product with a higher tendency to form aggregates during storage or use. Although the PRCA problem is under control, unbiased explanations of the effect of the formulation change are important now that protein therapeutics are becoming a major part of the new drugs introduced.

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Potential impact and cost-effectiveness of Golden Rice

To the editor

A News & Views article by Michael Grusak in last year's April issue (*Nat. Biotechnol.* **23**, 429–430, 2005) highlighted the unresolved debate concerning the efficacy of Golden Rice in addressing the problem of vitamin A deficiency (VAD). He pointed out that an assessment of the potential impact of Golden Rice on this type of malnutrition requires the consideration of multiple variables, including the target individuals' life stages, the average amount of rice consumed daily by these individuals and the percentage of β-carotene that would be absorbed from rice. He further explains how early critics of the original Golden Rice technology had used simple estimates of these variables to suggest that unrealistic amounts of the transgenic rice would need to be consumed to satisfy the recommended dietary intakes of vitamin A equivalents (exclusively) through rice consumption. By replacing the daffodil phytoene synthase gene with the equivalent gene from maize, researchers have managed to increase the amount of β-carotene that accumulates in rice considerably[1]. However, a sound impact analysis of this new Golden Rice 2 variety, based on a solid methodological framework, is still outstanding.

Previous impact studies of Golden Rice either focused solely on effects of the rice on individual β-carotene intakes without considering the outcome on the health of those suffering from VAD[2] or considered health outcomes but used only highly aggregate intake data without taking into account important nutritional features like dietary heterogeneity[3]. Using a methodology developed for comprehensive *ex ante* evaluation, we present here a framework that substantially improves on these studies by combining health and nutrition details, as well as socioeconomic and policy factors, thus increasing the reliability of the results.