parenteral product packaging scientists tend to define critical leak size as any defect that might theoretically allow the ingress of even one microorganism under the most severe challenge conditions. With this in mind, the following discussion of pharmaceutical sciences research addressing critical leaks is offered.

Direct Comparison of Microbial Ingress to Physicochemical Leak Tests

In the late 1980s, researchers led by Dana Morton designed a series of tests to determine the gaseous, liquid, and microbial barrier properties of the classic parenteral vial package, that is, vial/closure compression seal systems (7,8). Test packages consisted of a simulated vial, fashioned from stainless steel, stoppered with disc-shaped closures made of various elastomers, either uncoated or laminated with various fluorocarbon or polypropylene-based polymeric materials, and sealed at a range of capping forces. Test packages were mounted onto a manifold equipped with a differential pressure transducer. The manifold with test package was pressurized with filtered nitrogen to an initial target value. Package leakage rate was measured by monitoring the test system's pressure drop over time. With this device, various elastomeric closures applied to the test vial across a wide range of compression forces were tested for their ability to affect a seal. Measured gas flow rates ranged from 10^{-3} to 10^{-7} Pa $\cdot$ m^3 $\cdot$ sec^{-1} (or 10^{-2} to 10^{-6} cc/sec) at 3 psig differential pressure test conditions.

After gaseous leakage rate determination, test packages were transferred to a separate test manifold for evaluating the seal's microbial barrier properties. Test packages, filled with a saline lactose broth suspension of *Pseudomonas aeruginosa*, were inverted so that the finish area was immersed in sterile saline. The test packages were pressurized to 3 psig for 15 minutes, replicating the differential pressure decay test conditions. *P. aeruginosa* migration from the package into the immersion fluid was determined using a filter plate count method. *P. aeruginosa* was shown incapable of passing through vial/closure compression seals that exhibited gas leakage rates of less than 10^{-5} Pa $\cdot$ m^3/sec (or 10^{-4} std cc/sec). Interestingly, there were vials that failed to allow microbial ingress even at gaseous leakage rates significantly higher than the critical leakage cut-off rate.

In the same publication, Morton determined the likelihood of liquid leakage across the test package seal. Test vials mounted on a manifold were filled with aqueous copper sulfate solution, and immersed, closure-end down, into distilled water. After pressurization for 15 minutes at 3 psig, copper sulfate presence in the water was measured by atomic absorption. The test was calculated to be capable of detecting as little as 0.1 μL of copper solution in the immersion water. No packages of gas leak rates less than 10^{-5} Pa $\cdot$ m^3 $\cdot$ sec^{-1} demonstrated microbial or liquid tracer leakage. Interestingly, liquid passage occurred for every package exhibiting gas leakage at or above this rate limit, while microbial leakage only occurred sporadically, with the number of colony forming units moving across the seal bearing no relation to the gas flow rate.

Later, Kirsch et al. worked to correlate helium leakage rate to the probability of microbial ingress using a liquid challenge media (9,10). While Morton et al. investigated leakage across a vial/closure compression seal, Kirsch and team studied leakage through glass micropipettes of various sizes imbedded in the walls of glass vials. A population of vials containing leak paths ranging in nominal diameter from 0.1 to 10 μm was flooded with helium and subsequently tested for helium leak rate using a mass spectrometry leak rate detector. These same vials were then filled with sterile media, and immersion challenged for 24 hours at 35°C with a saline lactose suspension of 10^8 to 10^{10} colony forming units of *B. diminuta* (*Brevundimonas diminuta*) and *Escherichia coli*, additionally spiked with magnesium ion tracer. Prior to challenge, test vials were thermally treated to eliminate airlocks within the micropipette lumen and establish a liquid path between the microbial challenge media and the test units' contents. After immersion, test vials were incubated at 35°C for an additional 13 days.

Kirsch's results showed that microbial ingress probability decreased as hole size and helium leakage rate decreased. Yet even under such extreme challenge conditions, only 3 of 66 test vials with log leak rates less than −4.5 std cc/sec failed the microbial challenge, consistent with the vial/closure interface leakage results reported by Morton. The probability of microbial ingress dramatically dropped from over 60% to about 10% within the helium log leak rate range of log −3.8 to −4.5 std cc/sec, which roughly corresponds to a leak nominal diameter of 0.4 to 1.0 μm. No ingress occurred through holes of helium leakage rate between 10^{-5} and $10^{-5.8}$ std cc/sec.